

# PLANT PHYSIOLOGY

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## ERRATA

### VOLUME 14

Page 5, line 21, delete comma after word wheels.

Page 92, citation 7, first line, for "The" read its.

Page 139, line 9, for "necessary alternatively" read necessary. Alternatively.

Page 142, line 21, for "equation (9)" read equation (19).

Page 187, line 14, for "EDWARD" read EDWIN.

Page 280, last line of legend, for "gallon" read gallon can.

Page 418, line 20, for "is" read it.

Page 445, line 4 of summary, for "effect" read effects.

Page 459, line 13, for "were" read was.



THOMAS ANDREW KNIGHT  
AUGUST 12, 1759—MAY 11, 1838



# PLANT PHYSIOLOGY

JANUARY, 1939

THOMAS ANDREW KNIGHT  
IN MEMORIAM

CHARLES A. SHULL AND J. FISHER STANFIELD

(WITH ONE PLATE<sup>1</sup> AND ONE FIGURE)

During the latter part of the eighteenth century the physiological behavior of plants challenged the attention of many investigators. The brilliant discoveries of PRIESTLY, INGEN HOUSZ, SENEBIER, and DE SAUSSURE were great historical events, and their published works form an impressive and unforgettable chapter in the history of plant physiology. To this galaxy of stellar performers must be added the name of THOMAS ANDREW KNIGHT, who, beginning his investigations during the decade from 1780 to 1790, became subsequently one of the keenest and most prolific students of plant physiology and horticulture. His publications in the *Philosophical Transactions of the Royal Society of London* began in 1795, and more than 20 papers appeared in the *Transactions* up to the year 1828. In addition he published well over 100 papers in horticulture, and several well known independent treatises. His career was brought to a close by death in 1838, at the age of 79 years.

Because he lived during a reactionary period, his work has never been fully appreciated. It seems most fitting, therefore, with the passage of a century since his death, to look back through the years with appreciation and understanding, especially understanding of the primitive state of knowledge throughout the realm of science, to appraise his work, and to commemorate his services to the science which he loved and his great practical services to mankind. His name will forever be associated with, and his work a part of, the history of biological science during the early decades of the nineteenth century.

<sup>1</sup> The plate has been reproduced from a photograph of an original in the private portrait collection of Dr. R. B. Harvey of the University of Minnesota. We wish to express to him our thanks and appreciation for the privilege of reproducing this fine portrait.

The first of his many contributions was entitled *Observations on the Grafting of Trees*, which was written at Elton, Herefordshire, April 13, 1795, as a letter to his friend and adviser, SIR JOSEPH BANKS, who read the letter before the Royal Society on April 30, 1795. The paper discussed the inheritance of decay among fruit trees, and the propagation of debility by grafting.

Encouraged by the reported reception of this paper, he continued, even long after he had been elected a Fellow of the Royal Society, to send his papers to Sir JOSEPH to be read. He was innately shy and modest, often apologetic for his shortcomings in "not being very deeply read in the experiments which naturalists have made on plants" and this may account for the presentation of his reports as letters to his intimate friend.

In appraising his work at the annual meeting of the Royal Society following his death, His Royal Highness the DUKE of SUSSEX, speaking as retiring president, rated the first paper published in 1795 as one of the most valuable of his contributions. This rating was based, no doubt, upon its practical aspects, because it dealt with the problem of "canker and moss," and the propagation of these diseases through grafts or layers to new trees. Plant physiologists, however, attach greater significance to his more scientific studies of gravitational and centrifugal action upon the roots and tops of plants. Many of his papers contain very valuable observations which one wishes were the possession of every student of plant physiology. Lord SUSSEX said: "Mr. Knight was a person of great activity of body and mind and of singular perseverance and energy in the pursuit of his favorite science: he was a very kind and agreeable writer, and it *would be difficult to name any other contemporary author in this or other countries who has made such important additions to our knowledge of horticulture and the economy of vegetation.*" (Italics ours.)

An examination of his papers reveals the truth of the president's estimate. The second paper, *An Account of Some Experiments on the Fecundation of Vegetables*, published in the Transactions in 1799, contains an account of some experiments on peas which he began in 1787. Interested mainly in the improvement of apples, he realized that results would be slow with perennial and slow-to-fruit varieties; he therefore decided to use annuals to obtain information more rapidly as to the effects of fertilization. Speaking of annuals he says: "Amongst these, none appeared so well calculated to answer the purpose as the common pea; not only because I could obtain many varieties of this plant of different forms, sizes, and colours; but also, because the structure of its blossoms, by preventing the ingress of insects and adventitious farina, has rendered its varieties remarkably permanent."

He records the use of the farina (pollen) of a gray pea in fertilizing a white variety. The seeds resulting from the original fertilization were not modified in appearance, but when these  $F_1$  seeds were planted, all of the offspring produced plants with nothing but dark gray seeds. Then he says: "By introducing the farina of another white variety (or, in some instances by simple culture) I found this colour was easily discharged, and a numerous variety of new kinds produced, many of which were, in size, and in every other respect, much superior to the original white kind, and grew with excessive luxuriance, some of them attaining a height of more than twelve feet." Here we see KNIGHT observing dominance, recessive behavior, and heterosis almost 80 years before MENDEL's day. With a more complete understanding, and quantitative analysis of his results in succeeding generations, KNIGHT might easily have occupied the position in genetics now given to MENDEL. One wonders how aware MENDEL may have been of this interesting work carried on by KNIGHT nearly a century earlier; for KNIGHT had the virtue not to place his work in an obscure journal.

He turned his attention to ascent and descent of sap in trees, and showed himself a true disciple of HALES and DUHAMEL. In tracing the ascent of sap he used a deep-colored infusion of grape skins to locate the translocating tissues, and also used ringing technique for studies of sap descent in the phloem. He was always critical in his judgments, and had a fine sense of disagreement with previous authors, and with the current beliefs of his day. He says: "In the authors I have looked into, I have seen many contradictory experiments related, and many conclusions drawn from a small number of facts, and I have found much that does not well agree with the things that have come under my own observation." Speaking of leaves he says: "This organ has much engaged the attention of naturalists, particularly Mr. BONNET: but their experiments have chiefly been made on leaves severed from the trees; and, therefore, whatever conclusions have been drawn, stand on very questionable ground." His papers are full of such critical statements.

In a paper on "*Experiments and Observations on the Motion of the Sap in Trees*" read in 1804, he mentions the eleventh plate in HALES' *Vegetable Staticks* disparagingly: "The experiments and still more, the Plates of HALES, have induced naturalists to draw conclusions in direct opposition to the preceding. But the Plates of that great naturalist are not always taken correctly from nature; and Plates, under any circumstances, however fair and candid the intentions of an author may be will too often be found somewhat better calculated to support his own hypotheses, than to elucidate the facts he intends to state." KNIGHT praises DUHAMEL highly for his accuracy, overlooking the fact, apparently, that DUHAMEL copied HALES' eleventh plate for his own work published



in 1758, and accepted it without question. Later KNIGHT was compelled to admit his own errors in connection with gravitational studies of sap movement in reversed stems.

The great paper on the influence of gravitation and centrifugal force on the responses of seedlings was read before the Royal Society in 1806. It bore the title: "*On the Direction of the Radicle and Germen during the Vegetation of Seeds.*" The paper was not illustrated, but it is a classic of simplicity and clarity. The figure of his wheels presented in this paper is taken from Sir HUMPHREY DAVY's *Agricultural Chemistry*, published in 1813. We quote the essential paragraphs as follows: "I conceived that if gravitation were the cause of the descent of the radicle, and of the ascent of the germen, it must act either by its immediate influence on the vegetable

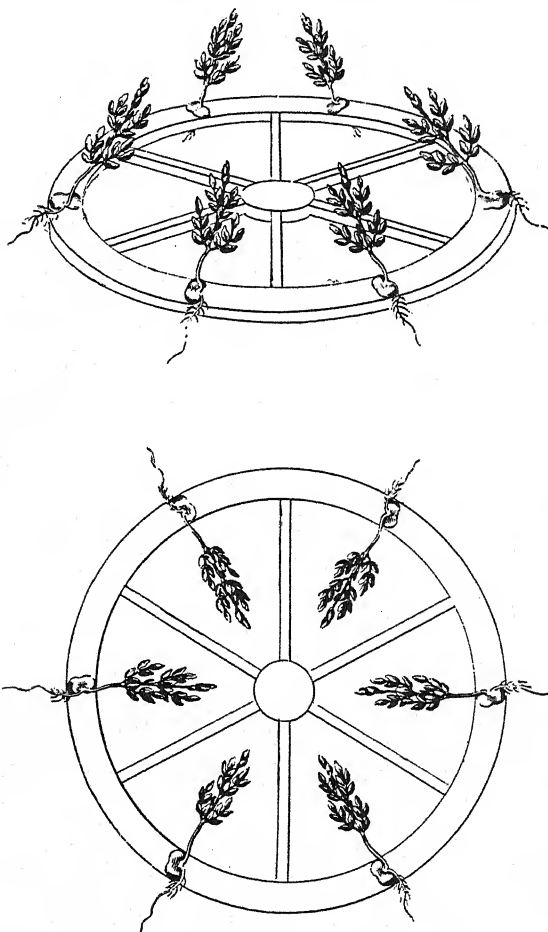


FIG. 1. Diagrammatic representation of KNIGHT's rotating wheels, showing the direction of growth. From DAVY's *Agricultural Chemistry*.

fibres and vessels during their formation, or on the motion and consequent distribution of the true sap afforded by the cotyledons: and as gravitation could produce these effects only whilst the seed remained at rest, and in the same position relative to the attraction of the earth, I imagined that its operation would become suspended by constant and rapid change of the position of the germinating seed, and that it might be counteracted by the agency of centrifugal force."

"Having a strong rill of water passing through my garden, I constructed a small wheel similar to those used for grinding corn, adapting a wheel of different construction, and formed of very slender pieces of wood, to the same axis. Round the circumference of the latter, which was eleven inches in diameter, numerous seeds of the garden bean, which had been soaked in water to produce their greatest degree of expansion were bound, at short distances from each other. The radicle of these seeds were made to point in every direction, some towards the center of the wheel, and others in the opposite direction; others as tangents to its curve, some pointing backwards, and others forwards, relative to its motion; and others pointing in opposite directions in lines parallel with the axis of the wheels. The whole was inclosed in a box, and secured by a lock, and a wire grate was placed to prevent the ingress of any body capable of impeding the motion of the wheels. The water being then admitted, the wheels performed something more than 150 revolutions in a minute; and the position of the seeds relative to the earth was of course as often perfectly inverted, within the same period of time, by which I conceive that the influence of gravitation must have been wholly suspended."

"In a few days the seeds began to germinate, and as the truth of some of the opinions I had communicated to you, and of many others which I had long entertained, depended on the result of the experiment, I watched its progress with some anxiety, though with not much apprehension; and I had soon the pleasure to see that the radicles, in whatever direction they were protruded from the seed, turned their points outward from the circumference of the wheel, and in their subsequent growth receded nearly at right angles from its axis. The germens, on the contrary, took the opposite direction, and in a few days their points all met at the centre of the wheel. Three of these plants were suffered to remain on the wheel, and were secured to its spokes to prevent their being shaken off by its motion. The stems of these plants soon extended beyond the centre of the wheel; but the same cause, which first occasioned them to approach its axis, still operating, their points returned and met again at its centre."

"The motion of the wheel being in this experiment vertical, the radicle and germen of every seed occupied, during a minute portion of time in each revolution, precisely the same position they would have assumed had

the seed vegetated at rest; and as gravitation and centrifugal force also acted in lines parallel with the vertical motion and surface of the wheel, I conceived that some slight objections might be urged against the conclusions I felt inclined to draw. I therefore added to the machinery I have described another wheel which moved horizontally over the vertical wheels; and to this, by means of multiplying wheels of different powers, I was enabled to give many different degrees of velocity. Round the circumference of the horizontal wheel, whose diameter was also eleven inches, seeds of the bean were bound as in the experiment, which I have already described, and it was then made to perform 250 revolutions in a minute. By the rapid motion of the water wheel much water was thrown upwards on the horizontal wheel, part of which supplied the seeds upon it with moisture, and the remainder was dispersed, in a light and constant shower, over the seeds in the vertical wheel, and on others placed to vegetate at rest in different parts of the box."

"Every seed on the horizontal wheel, though moving with great rapidity necessarily remained in the same position relative to the attraction of the earth; and therefore the operation of gravitation could not be suspended, though it might be counteracted, in a very considerable degree, by centrifugal force; and the difference, I had anticipated, between the effects of rapid vertical and horizontal motion soon became sufficiently obvious. The radicles pointed downwards about ten degrees below, and the germens as many degrees above, the horizontal line of the wheel's motion; centrifugal force having made both to deviate 80 degrees from the perpendicular direction each would have taken, had it vegetated at rest. Gradually diminishing the rapidity of the motion of the horizontal wheel, the radicles descended more perpendicularly, and the germens grew more upright; and when it did not perform more than 80 revolutions in a minute, the radicle pointed about 45 degrees below, and the germen as much above, the horizontal line, the one always receding from, and the other approaching to, the axis of the wheel.

"I would not, however, be understood to assert that the velocity of 250, or of 80 horizontal revolutions in a minute will always give accurately the degrees of depression and elevation of the radicle and germen which I have mentioned; for the rapidity of the motion of my wheels was sometimes diminished by the collection of fibres of conferva against the wire grate; which obstructed in some degree the passage of the water: and the machinery, having been the workmanship of myself and my gardener, cannot be supposed to have moved with all the regularity it might have done, had it been made by a professional mechanic. But I conceive myself to have fully proved that the radicles of germinating seeds are made to descend, and their germens to ascend, by some external cause, and not by any power

inherent in vegetable life: and I see little reason to doubt that gravitation is the principal, if not the only agent employed, in this case, by nature."

In still others of his papers we find him solving problems that are refreshingly modern. One of them "*On the Inverted Action of the Alburnous Vessels of Trees*" contains an account of the profuse flowering even of very early varieties of potatoes, which ordinarily do not blossom, when he prevented the consumption of the carbohydrates by suppressing stolon formation. He accomplished the suppression in an ingenious fashion. Those who have given attention to the influence of clipping on forage survival and succeeding yields will appreciate this statement from a paper read in 1805: "I have constantly found, in my practice as a farmer, that the produce of my meadows has been immensely increased when the herbage of the preceding year had remained to perform its proper office till the end of the autumn, on ground which had been mowed early in the summer." And the relation of girdling to blossom-bud setting in apples receives the following comment: "It has been long known to gardeners that taking off a portion of bark round the branch of a fruit tree occasions the production of much blossom on every part of that branch in the succeeding season." If he had mentioned the high-carbohydrate content of these tissues he would truly have been a hundred years ahead of his time.

His breadth of interest is indicated by the fact that he wrote not only about many kinds of fruits and vegetables, but about insects, such as aphids and bees; and various plant diseases, such as blights, cankers, etc. He even interested himself in the formation of ice in the bottoms of rivers.

Born at Wormsley Grange, near Hereford, on August 12, 1759, son of THOMAS KNIGHT and grandson of RICHARD KNIGHT who won a fortune as an iron master, THOMAS ANDREW was educated at Ludlow School, later at Chiswick, and finally at Oxford as a matriculant in Baliol College. He was 20 years old at the time he entered Oxford. He was possessed of an inherent interest in all kinds of plants and animals and was an eager sportsman and excellent shot. He utilized these inclinations, however, only as an opportunity for studying nature. The facts and incidents collected at this early period contributed a fund of information which formed the basis of many of his subsequent investigations; and his possession of land and funds gave him every opportunity to follow his natural inclinations. After leaving Oxford he lived at Elton where all of his early papers were written. The last of his letters to Sir JOSEPH BANKS from Elton was read on February 23, 1809. The next succeeding contribution, read on June 22, 1809 was sent from Downton Castle, where THOMAS ANDREW had an estate of ten thousand acres which was deeded to him by his older brother, RICHARD PAYNE KNIGHT, who is best known as a numismatist.

While still at Elton, KNIGHT helped to found the Royal Horticultural Society in 1804 as a charter member, and became its president in 1811; this position he held for 27 years, until his death in 1838. He became a fellow of the Royal Society in 1805, was awarded the Copley medal in 1806, and was elected a Fellow of the Linnean Society in 1807. He was also awarded the first Knightian Medal of the Royal Horticultural Society, a medal that bears his portrait and which was founded and named in his honor. In addition to those mentioned above he received nine other medals and was a member of 20 Societies in various countries. All of these honors were richly deserved.

In 1791, at the age of 33, he married FRANCES FELTON whose gentleness of disposition and unceasing endeavor to promote his comfort and happiness was a major factor in his achievements. They had four children, three daughters and one son. The latter was accidentally killed when near his 32nd year; this incident had a profound effect upon KNIGHT's thought and work for many years. His wife and daughters survived him at his death.

Anyone who reads THOMAS ANDREW KNIGHT's scientific contributions, his treatise *On the Manufacture of Cider and Perry* (1797), *A Treatise on the Culture of the Apple and Pear* (1797), *Pomona Herefordiensis* (1811), and his numerous practical papers on horticultural practices, must be impressed with his keen intellect, his unusual powers of observation, and his ability to express himself with force and clarity. His singular powers of memory, which he retained until his death, were of particular note. His skill in all kinds of horticultural operations, and his appreciation of the problems inherent in the production of fruits and vegetables, turned him to very practical services. One of his peculiarities was the readiness by which, with his own hands and the assistance of a common carpenter or blacksmith, he would construct all of the machinery required in his most elaborate experiments.

He placed England's horticulture on a solid foundation, and its subsequent growth and development is an enduring monument to his genius. As the years pass away, his memory will be preserved in the hearts and minds of all who are able to appreciate the time in which he lived. He stands as one of the most important contributors to our knowledge of natural history during the early part of the 19th century.

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# ANTAGONISM OF CERTAIN ELEMENTS ESSENTIAL TO PLANTS TOWARD CHEMICALLY RELATED TOXIC ELEMENTS

ANNIE M. HURD-KARRER

(WITH FIVE FIGURES)

The hypothesis developed to explain the quantitative aspects of selenate-sulphate antagonism was based on the assumption of unselective absorption and utilization of the two ions by virtue of their chemical similarity (18, 19). The idea subsequently received support from plant analyses showing that the amount of selenium taken into the tissues of certain plants parallels that of sulphur, as though selenium absorption were determined by the sulphur requirement of the species (20). Reasoning by analogy to other pairs of chemically related elements, one of which is toxic and the other essential in nutrition, has led to the discovery that arsenate toxicity is a function of phosphate availability, rubidium toxicity of potassium availability, and strontium toxicity of calcium availability. In view of the small number of pairs that can be selected on such a basis, positive results with all three are highly suggestive of the validity of the basic assumption. The present paper presents the experimental evidence on which the previous brief reports of these relationships were based (22, 23).

## Experimentation

### INHIBITION OF ARSENIC TOXICITY BY PHOSPHORUS

Because their positions in the periodic table of elements are analogous to those of sulphur and selenium in an adjoining group, phosphorus, an essential element capable of being absorbed in relatively large amounts, and arsenic, a highly toxic element, chemically much like phosphorus and adjacent to it in division B of group V of the periodic table (16, p. 203), were chosen for experimentation.

The most obvious effect of arsenic on wheat was killing of the roots with withering and stunting of the tops, apparently as a result of root injury (fig. 1, D). The severely damaged leaves were narrow and stiff, and sometimes pale green in color.

The effect of phosphates on these symptoms was studied first in water cultures and then in several kinds of soil.

WATER CULTURES.—Seedlings of Hard Federation wheat were grown for several weeks in low-, medium-, and high-phosphate solutions containing disodium arsenate ( $\text{Na}_2\text{HAsO}_4 \cdot 7 \text{H}_2\text{O}$ ). The three nutrient solutions, with 10, 60 and 120 parts per million of phosphorus, contained, in 19 liters, 31, 184, and 367 cc.  $\text{M}/10 \text{CaH}_4(\text{PO}_4)_2 \cdot \text{H}_2\text{O}$ , respectively; plus 95 cc.  $\text{M}/5 \text{MgSO}_4$ ,



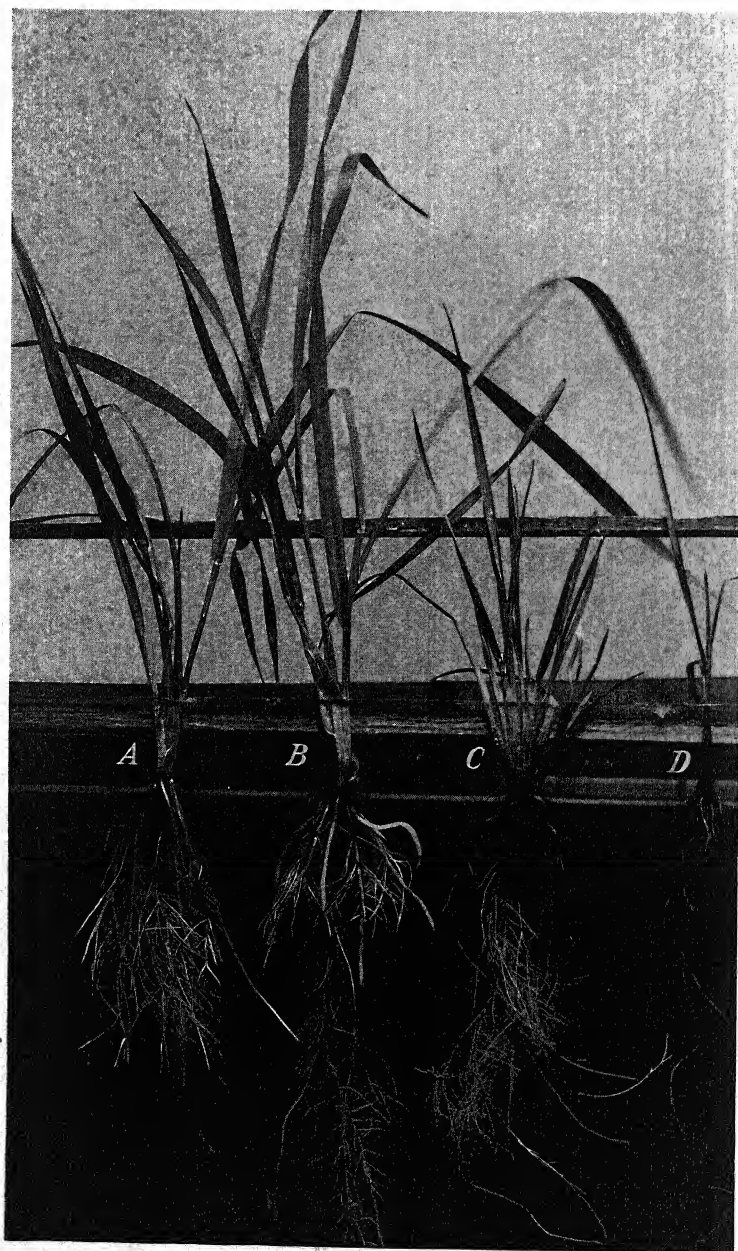


FIG. 1. Wheat plants injured by rubidium (B), strontium (C), and arsenic (D), in comparison with control plant (A).

75 cc. M/1  $\text{NH}_4\text{NO}_3$ , 60 cc. M/2 KCl, and 100 cc. M/60 ferric citrate. To equalize the differences in calcium, the first two of these solutions had, in addition, 67 and 37 cc. M/2  $\text{CaCl}_2$ , respectively. They thus differed with respect to chlorine as well as phosphate; but the former did not affect the results, for it was highest in the low-phosphate solution, where it was shown by control cultures to be non-toxic.

The pH values of the low- and high-phosphate solutions were initially 6.6 and 5.7, produced by the addition of one and two drops, respectively, of sodium hydroxide to a 600-cc. culture.<sup>1</sup> After three weeks' growth of the seedlings in the solutions containing arsenic, the corresponding values were 5.7 and 5.2. These values seemed sufficiently similar to preclude any possibility that the differences in arsenic injury were due to acidity differences. Moreover, the same results were obtained when the initial pH value of the high-phosphate solution was brought to 6.5.

Each culture consisted of three seedlings in a flask containing 600 cc. of nutrient solution. Control cultures without arsenic were a part of each experiment, and showed that damage to the plants in the low-phosphate solutions containing arsenic was actually due to the arsenic in each case and not to phosphate deficiency.

That the degree of arsenic toxicity depended on the phosphate concentration in the nutrient solution is illustrated by the experiment of figure 2. On comparing the effects of a given absolute amount of arsenic at the three phosphate levels, it can be seen, for example, that the plants were almost killed by 30 p.p.m. of arsenic where there were only 10 p.p.m. of phosphorus available, slightly stunted with 60 p.p.m. of phosphorus, and uninjured with 120 p.p.m. The minimum arsenic concentrations causing extreme stunting were 10, 60, and 120 p.p.m. with phosphorus concentrations of 10, 60 and 120 p.p.m. respectively. This high degree of toxicity thus resulted from a 1:1 arsenic/phosphorus ratio irrespective of the absolute concentrations involved. In general, the arsenic was definitely toxic in the presence of less than four times as much phosphorus, but non-toxic where there was more than four times as much.

Injury was somewhat greater at a given ratio in the high-phosphate than in the low-phosphate series. That is, injury at a given ratio increased slightly with increasing concentration of arsenic.

Other experiments were carried out with both wheat and barley in solutions containing various concentrations of phosphate obtained by mixing,

<sup>1</sup> To guard against possible complicating effects of the difference in sodium necessitated by the pH adjustment, the low-phosphate cultures were duplicated in one experiment with the larger quantity of sodium hydroxide (2 drops) added to each. Arsenic toxicity proved to be of the same degree as that in the corresponding cultures with the smaller addition of sodium hydroxide, indicating that the effects attributed to phosphate differences were not due to the small difference in sodium concentration.





FIG. 2. Comparative toxicity of disodium arsenate to wheat in three different nutrient solutions, containing 10, 60 and 120 p.p.m. of phosphorus, respectively. The cultures in vertical alignment show the effect of a given As/P ratio. (Figures under flasks denote

TABLE I

THE TOXICITY OF SODIUM ARSENATE TO WHEAT AND BARLEY SEEDLINGS GROWN THREE WEEKS  
IN NUTRIENT SOLUTIONS OF DIFFERENT PHOSPHATE CONCENTRATIONS

EXPERIMENT	GREEN WEIGHT OF THREE PLANTS GROWN WITH PHOSPHORUS				
	10 p.p.m.	37.5 p.p.m.	65 p.p.m.*	92.5 p.p.m.	120 p.p.m.
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
1. Wheat					
Arsenic, 30 p.p.m. ...	0.50	.....	1.15	.....	2.16
Arsenic, 15 p.p.m. ...	0.75	.....	1.91	.....	2.51
Arsenic, none .....	2.67	.....	2.58	.....	2.38
2. Wheat					
Arsenic, 12.5 p.p.m.	0.70	.....	4.34	.....	4.78
Arsenic, none .....	3.75	.....	3.60	.....	5.12
3. Barley (fig. 3, A)					
Arsenic, 12.5 p.p.m.	0.38	.....	5.92	.....	6.21
Arsenic, none .....	6.08	.....	5.69	.....	6.34
4. Wheat (fig. 3, B)					
Arsenic, 30 p.p.m. ...	0.62	0.88	1.67	2.60	2.59
Arsenic, none .....	3.43	3.54	3.54	3.58	3.08

\* This concentration was 60 p.p.m. instead of 65 p.p.m. in the first experiment.

in proper proportions, the low- and high-phosphate solutions. The data given in tables I and II, and the illustrations included in figure 3, all show the inverse relation between arsenic toxicity and phosphate concentration. Table II shows that the arsenic generally produced a greater reduction in growth at a given arsenic/phosphorus ratio (in comparison with corresponding controls without arsenic) when the solutions were renewed weekly than when they were unchanged.

TABLE II

THE TOXICITY OF 12 P.P.M. ARSENIC (AS SODIUM ARSENATE) TO ONE-MONTH-OLD WHEAT  
SEEDLINGS IN NUTRIENT SOLUTIONS OF DIFFERENT PHOSPHATE CONCENTRATIONS, WITH AND WITHOUT WEEKLY RENEWAL

PHOSPHATE AS P	APPROXIMATE AS/P RATIO	GREEN WEIGHT OF THREE PLANTS			
		SOLUTIONS NOT RENEWED		SOLUTIONS RENEWED WEEKLY	
		WITH ARSENIC	WITHOUT ARSENIC (CONTROLS)	WITH ARSENIC	WITHOUT ARSENIC (CONTROLS)
<i>p.p.m.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
65	1: 5	2.43	2.44	2.40	3.21
51	1: 4	2.34	2.23	2.51	2.89
37	1: 3	1.82	2.68	2.53	3.05
24	1: 2	1.42	2.17	1.50	2.40
10	1: 1	0.78	2.48	0.79	2.65

It is concluded that under the conditions of these various experiments, a 1:1 arsenic/phosphorus ratio was almost lethal, a 1:2 ratio highly toxic, while a 1:5 ratio rendered the arsenic harmless. Because of the probability that some of the phosphate was precipitated as ferric phosphate, especially at the higher concentrations, these ratios are considered approximations only. However, they have been sufficiently constant for a given degree of injury to permit the conclusion that arsenic injury is determined by proportionate phosphate availability.

SOIL CULTURES.—Hard Federation wheat was grown in the greenhouse in 10-inch pots containing 6 kg. of soil on a layer of gravel. The arsenic as  $\text{Na}_2\text{HAsO}_4 \cdot 7\text{H}_2\text{O}$  and the phosphate as  $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$  were added in solution, thoroughly stirred into the soil of each individual pot.

The first experiments were with the clay loam (Keyport) of the Arlington Experiment Farm, Rosslyn, Virginia. Improvement in the growth of the arsenic-injured plants was noted in pots with excess phosphate when the plants entered the jointing stage although it had not been apparent in younger stages. When the plants were cut after nine weeks' growth it was found that the toxicity of the arsenic was definitely reduced by the phosphate applications, as shown by somewhat higher dry weights and unretarded stages of development. The averaged plant weights showed that 125 and 250 p.p.m. of arsenic reduced yields to 54 and 20 per cent., respectively, of that of the controls. With phosphate applications corresponding to 125 p.p.m. of P, these yields were increased to 73 and 41 per cent. of those of similarly treated controls without arsenic.

The experiment was repeated with a sandy loam<sup>2</sup> in the expectation of more positive results in soil of lesser adsorptive capacity. The growth of the plants with several different concentrations of arsenate and phosphate is indicated in table III. In every case the addition of phosphate to the soil

TABLE III

THE EFFECT OF SODIUM PHOSPHATE ON THE TOXICITY OF SODIUM ARSENATE TO WHEAT (2 MONTHS OLD) IN A SANDY LOAM SOIL

ARSENIC	WEIGHTS OF 13 PLANTS WITH THE FOLLOWING CONCENTRATIONS OF PHOSPHATE (P.P.M. P) ADDED TO SOIL		
	0	100 p.p.m.	200 p.p.m.
<i>p.p.m.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
0 .....	7.48	8.50	8.73
50 .....	4.09	8.53	8.08
100 .....	1.04*	3.14	6.30

\* These plants were in the pre-heading stage. All others were flowering.

<sup>2</sup> Obtained from an artificial fill from the Potomac River on the Arlington Experiment Farm, Virginia.

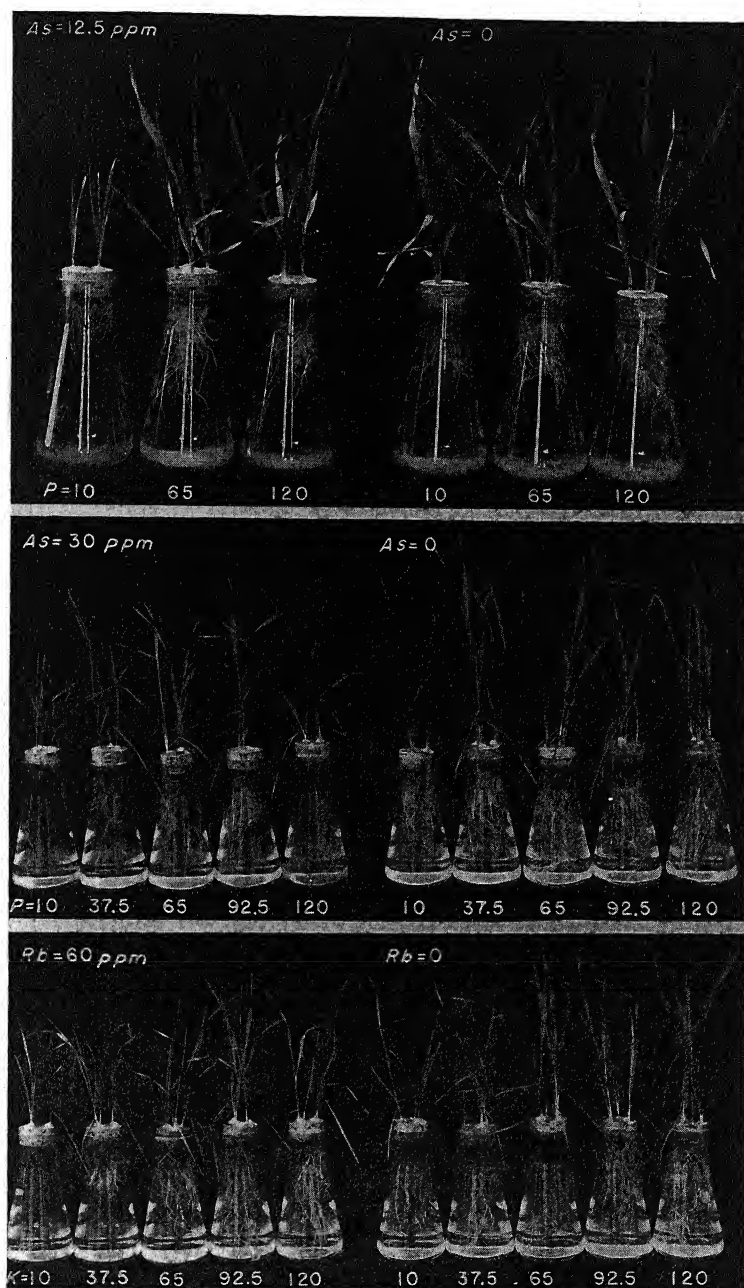


FIG. 3. Upper row: Effect of phosphate (10, 65, and 120 p.p.m. of P) on the toxicity of disodium arsenate (12.5 p.p.m. of As) to barley. Controls without arsenic to the right. Middle row: Effect of phosphate (10, 37.5, 65, 92.5, and 120 p.p.m. of P) on the toxicity of disodium arsenate (30 p.p.m. of As) to wheat. Controls without arsenic to the right.

greatly decreased the arsenic injury. The differences were apparent from the time the plants were but two weeks old.

In order to determine whether the progressive decrease in arsenic injury with increasing phosphate connoted a corresponding decrease in the arsenic taken into the leaves, the tops of these plants were analyzed<sup>3</sup> for arsenic. The series with 50 p.p.m. of arsenic gave the values 20, 18, and 15 p.p.m. for the phosphate levels of 0, 100, and 200 p.p.m. of P, respectively. With 100 p.p.m. of arsenic in the soil, the corresponding analyses were 15, 26, and 24 p.p.m. for the plants. The data thus show no significant effect of the phosphate on the amount of arsenic taken into the leaves. It is known that more arsenic accumulates in the roots than in the tops of plants (32, 39) so perhaps roots rather than tops should be analyzed in studying the arsenate-phosphate relation.

Another experiment comprised plants grown for five weeks in a still sandier soil, obtained from Gunston, Virginia, by Mr. J. D. REID of the Division of Soil Fertility. The arsenic proved to be so toxic in this soil that 50 p.p.m. practically killed the plants within a few weeks except where phosphate was added. One hundred p.p.m. of P definitely improved their condition, while 200 p.p.m. of P made them indistinguishable from the controls.

Thus the toxicity of sodium arsenate and the extent to which phosphate can inhibit it depend on the soil type. Apparently the explanation is to be found in the differing capacities of soils to adsorb arsenates and phosphates (10, 13, 38). GLE (13) has shown that the two are not adsorbed alike by a given soil, their relative adsorption depending on the nature of the soil colloids; for although phosphate seems to be adsorbed by both the aluminum and the iron of the colloids, arsenic is adsorbed chiefly by the iron.

In some South Carolina soils calcium arsenate injury is reported to be increased by phosphate, which acts apparently by combining with the iron in the soil, rendering it unavailable for insoluble combination with the arsenic (10). Other factors found to affect the toxicity of calcium arsenate in some of these soils are acidity, and reactive iron content. It is thus probable that the varying conditions for solubility, adsorption, and chemical combination of arsenic and phosphorus in soils render unpredictable the practical value of the arsenic-phosphorus antagonism. Most of the arsenic reaching the soil under field conditions is in the form of the comparatively insoluble calcium salt instead of the soluble sodium salt used in these experiments.

<sup>3</sup> The analyses were made by Mr. C. C. CASSIL with the Gutzeit method, through the courtesy of Dr. R. C. ROARK, Chief of the Division of Insecticide Investigations of the Bureau of Entomology and Plant Quarantine.

That the form in which the arsenic occurs is likely to be an important factor is suggested by the fact that sulphate more effectively reduces absorption of selenium from selenates than from selenites (21). By analogy, phosphates would be expected to have less effect on the toxicity of arsenites than on that of the arsenate used in these experiments.

It is obvious that the critical ratios determining growth in nutrient solutions do not apply directly to soils, as pointed out previously in connection with the selenium-sulphur antagonism. LIPMAN's critique of the "hypothesis of the lime-magnesia ratio" (26) is evidence of the difficulties and complications inherent in attempts to establish universally applicable ratios in soils.

#### INHIBITION OF RUBIDIUM TOXICITY BY POTASSIUM

Another pair of elements investigated comprised potassium, as the essential element, and rubidium, a moderately toxic element similar to potassium chemically and adjacent to it in division A of group I in the periodic table (16, p. 43).

The most characteristic symptom of rubidium injury was a peculiar thickening and stunting of the roots, especially of the crown roots (fig. 1, B). The leaves of severely injured plants were an abnormally dark green.

A preliminary experiment with a complete nutrient solution containing 94 p.p.m. of rubidium as rubidium chloride showed that the addition of an excess of potassium chloride to the cultures definitely reduced the toxicity of the rubidium. The green weights, in grams, of the three plants of duplicate cultures after three weeks were as follows:

	<i>With 60 p.p.m. of K</i>	<i>With 190 p.p.m. of K</i>
With 94 p.p.m. of rubidium .....	1.52	2.22
	1.57	2.16
Controls without rubidium.....	2.56	2.90
	2.71	2.45

In the second experiment the plants were grown with 100 p.p.m. of rubidium as rubidium chloride in solutions containing 10, 60, and 120 p.p.m., respectively, of potassium as KCl.<sup>4</sup> Rubidium injury was pronounced (especially that of the roots) with 10 p.p.m. of K, less with 60 p.p.m., and absent with 120 p.p.m. of K (fig. 4). The condition of the control plants showed that the injury ascribed to rubidium in the low-potassium cultures was not due to potassium deficiency.

<sup>4</sup> All ions except potassium and chlorine were equal in the three solutions. They each contained, in 19 liters, 184 cc. M/10 CaH<sub>4</sub>(PO<sub>4</sub>)<sub>2</sub>, 120 cc. M/1 NH<sub>4</sub>NO<sub>3</sub>, 95 cc. M/5 MgSO<sub>4</sub>, 37 cc. M/2 CaCl<sub>2</sub>, and 100 cc. M/60 iron citrate,—with 10, 59, and 117 cc. M/2 KCl for the low-, medium-, and high-potassium levels, respectively. The initial pH values of the three solutions were all between 3.9 and 4.1.

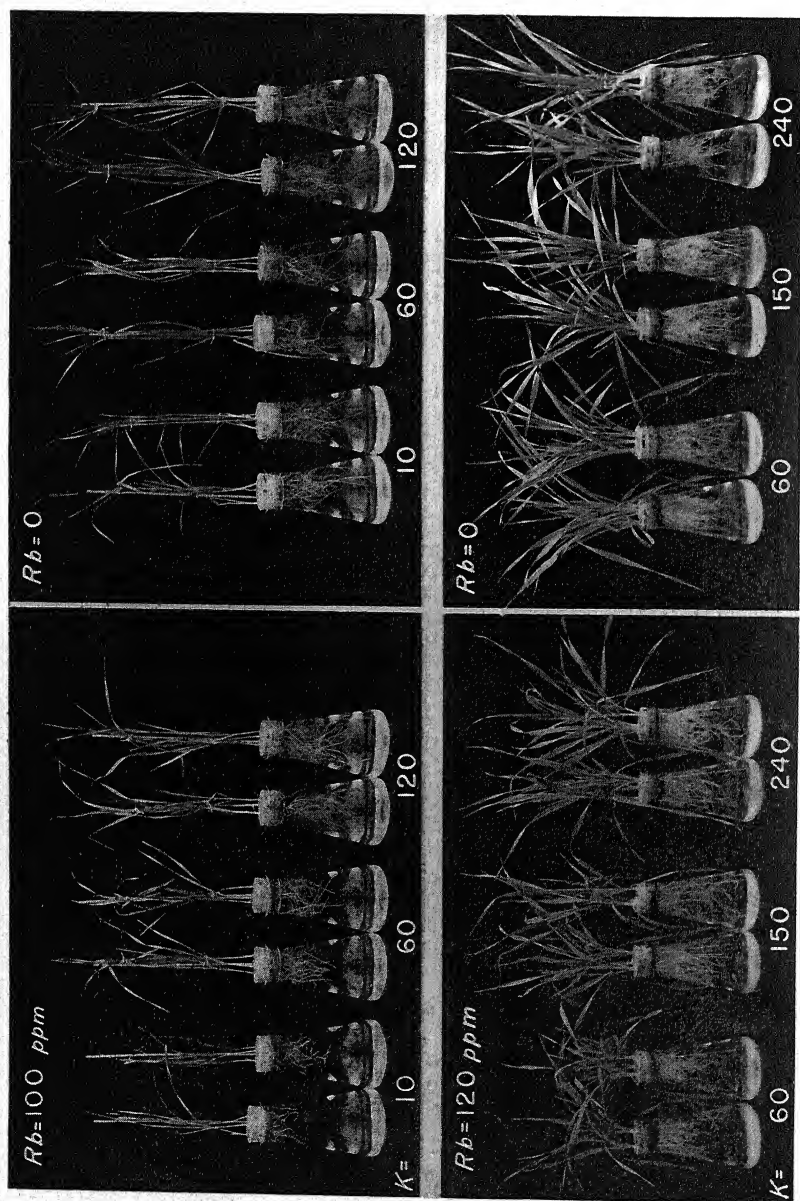


FIG. 4. Upper row: Effect of potassium (10, 60 and 120 p.p.m.) on the toxicity of rubidium chloride (100 p.p.m. of Rb) to wheat (duplicate cultures). Controls without rubidium to the right. Lower row: Effect of potassium (60, 150 and 240 p.p.m.) on the toxicity of rubidium chloride (120 p.p.m. of Rb) to barley (duplicate cultures). Controls without rubidium to the right.

Another experiment was set up with 60 p.p.m. of rubidium as rubidium chloride at five different potassium levels, obtained by mixing a low-potassium and a high-potassium solution in proper proportions.<sup>5</sup> Comparative degrees of rubidium injury to the plants are shown in figure 3 (lower row). The controls show that slight stunting of the tops resulted from potassium deficiency at the two lowest concentrations, 10 and 37.5 p.p.m., but the toxic effects of the rubidium in these solutions, especially those shown by the roots, were not obscured thereby. The rubidium injury was progressively reduced as the potassium in the solution increased, until with 92.5 p.p.m. the plants were as good as the corresponding controls without rubidium.

The experiment was repeated with 60 p.p.m. of rubidium in duplicate series of cultures, for one of which the solution was renewed every week, while for the other it was left unchanged for the duration of the experiment. The pH values were adjusted to 6.5, which was soon reduced to 4.3 where growth was vigorous and to 5.0 where it was not. The plants of both sets were cut after 6 weeks, at which time they were alike in showing rubidium injury in the low potassium solution and none where there was as much as 65 p.p.m. of potassium. An intermediate potassium concentration (37.5 p.p.m.) almost but not entirely inhibited the injury.

A final experiment was carried out with barley. Three potassium levels, 60, 150 and 240 p.p.m. as KCl were used, the lowest being made high enough to avoid symptoms of potassium deficiency.<sup>6</sup> The differences in chlorine were equalized with calcium chloride in duplicate series which provided incidentally an opportunity to determine whether calcium, known to have an ameliorating influence on a number of toxic conditions, might reduce rubidium toxicity and so refute the assumed specificity of the rubidium-potassium relation.

Since the potassium concentrations in these solutions were higher than in the preceding experiments, it was necessary to increase the rubidium concentration correspondingly in order to have a Rb/K ratio that would produce definite injury. So 120 p.p.m. of rubidium as RbCl was added to each culture except the controls.

<sup>5</sup> These two solutions contained the following salts, in 19 liters: 184 cc. M/10  $\text{CaH}_4(\text{PO}_4)_2$ , 75 cc. M/1  $\text{NH}_4\text{NO}_3$ , 95 cc. M/5  $\text{MgSO}_4$ , and 100 cc. M/60 iron citrate, with 10 and 117 cc. M/2 KCl, producing 10 and 120 p.p.m. of potassium, respectively, in the low- and high-potassium solutions. The acidity was adjusted to pH 6.4 with sodium hydroxide.

<sup>6</sup> The low- and high-potassium solutions both contained, in 19 liters, 184 cc. M/10  $\text{CaH}_4(\text{PO}_4)_2$ , 75 cc. M/1  $\text{NH}_4\text{NO}_3$ , 95 cc. M/5  $\text{MgSO}_4$ , and the iron citrate, the difference in potassium concentration being produced by 59 cc. and 234 cc. of M/2 KCl respectively. The intermediate potassium level was obtained by mixing the two solutions in equal proportions. The pH value of each was brought to 6.6 with a drop of a sodium hydroxide solution.



That this rubidium was toxic in the solution with 60 p.p.m. of potassium and non-toxic in that containing 150 p.p.m. is shown in figure 4. The flask to the left of each pair of cultures in these photographs is of the series in which chlorine differences were equalized by the addition of excess calcium chloride to the low-potassium solutions. Of interest is the fact that excess calcium (*cf.* the right-hand flask of the low-potassium pair) did not reduce rubidium toxicity.

#### INHIBITION OF STRONTIUM TOXICITY BY CALCIUM

Finally, the possibility of inhibiting the toxicity of strontium by calcium, an essential element of similar chemical properties (16, p. 56), was investigated. The two are adjacent in division A of group II of the periodic table.

Unlike the case with rubidium, strontium injury to the tops of the plants was always more pronounced than that to the roots. A curious effect frequently obtained was an increase in the number of tillers. With sufficiently high concentrations the plants were thick, short bunches of sometimes as many as twelve tillers (fig. 1, C) instead of the three to five normal ones of the control plants (fig. 1, A). Leaf symptoms, not always present on the young plants, were a mottled yellowish and grayish firing, first appearing at the tips.

Preliminary experiments showed strontium to be so slightly toxic that in order to obtain symptoms of injury in solutions containing enough calcium to prevent deficiency symptoms on the control plants, it was necessary to use very high concentrations. About 1000 p.p.m. of strontium as strontium chloride ( $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$ ) were required to kill the plants in a solution containing 45 p.p.m. of calcium as calcium nitrate.

The comparative growth of some plants grown with 500 and 1000 p.p.m. of strontium in solutions containing 45, 300, and 500 p.p.m. of calcium as  $\text{Ca}(\text{NO}_3)_2$ , all at pH 5.0 and with the nitrogen differences equalized by  $\text{NH}_4\text{NO}_3$ , is shown in table IV.

TABLE IV

THE TOXICITY OF STRONTIUM CHLORIDE TO WHEAT IN RELATION TO THE CONCENTRATION OF CALCIUM IN THE NUTRIENT SOLUTION

STRONTIUM	WEIGHTS OF THREE PLANTS WITH THE FOLLOWING CONCENTRATIONS OF CALCIUM		
	45 P.P.M.	300 P.P.M.	500 P.P.M.
<i>p.p.m.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
0 .....	9.04	8.35	9.18
500 .....	2.73	6.38	6.75
1000 .....	0.86	4.36	7.39

The extreme stunting produced by the strontium in the solution containing but 45 p.p.m. of calcium was markedly reduced by the higher calcium levels, but not entirely prevented. The reduction in injury was apparent not only from the plant weights but also from the inhibition of the leaf-tip firing which characterized the plants in the low-calcium solutions. Control plants showed the symptoms to be due to the strontium chloride and not to calcium deficiency.

In order to check the possibility that the toxic symptoms attributed to strontium might be due in part to the chlorine in the molecule, a series of plants was grown with sufficient potassium chloride to supply 414 p.p.m. of Cl, slightly more than was supplied by the strontium chloride in the 500-p.p.m. strontium solutions. The results were completely negative, the potassium salt producing no injury to the plants at any calcium level, and so justifying the assumption that the toxic symptoms produced by the strontium chloride were due to the strontium and not to the chlorine.

For the next experiment two nutrient solutions were made with 50 and 500 p.p.m. of calcium, respectively, as calcium nitrate, the nitrogen being equalized with ammonium nitrate.<sup>7</sup> An intermediate calcium concentration (275 p.p.m.) was obtained by mixing the two solutions in equal proportions. The strontium, 500 p.p.m., was added as the chloride in one series of triplicated cultures and as the nitrate in another. To check on possible effects of the anions, two other series were set up without strontium but with equivalent amounts of the chloride (405 p.p.m. of Cl) and nitrate (708 p.p.m. of  $\text{NO}_3$ ) of potassium.

Both the appearance of the plants (fig. 5) and their weights (table V) when cut after three weeks' growth in the solutions showed that 500 p.p.m. of strontium produced considerable stunting with 50 p.p.m. of calcium, very little with 275 p.p.m., and none with 500 p.p.m. The series with the chloride and nitrate of potassium instead of strontium showed that neither of the anions was involved, the plants being uninjured and like the controls at each calcium level. The control plants showed that there was no stunting or other abnormality from insufficient calcium in the low-calcium solutions, but that the high-calcium solution was somewhat toxic.

While these results appear to indicate that the toxicity of strontium is inhibited by an equivalent amount of calcium, the critical ratio has not been determined over a range of concentrations. At high concentrations, a rather heavy precipitate developed, rendering exact statement of ratios of doubtful value.

<sup>7</sup> Both contained, in 19 liters, 50 cc.  $\text{M}/5 \text{ K}_2\text{HPO}_4$ , 50 cc.  $\text{M}/5 \text{ KH}_2\text{PO}_4$ , 95 cc.  $\text{M}/5 \text{ MgSO}_4$ , and 100 cc.  $\text{M}/60$  iron citrate. The low-calcium solution contained 47.5 cc.  $\text{M}/2 \text{ Ca}(\text{NO}_3)_2$  and 214 cc.  $\text{M}/1 \text{ NH}_4\text{NO}_3$ ; the high-calcium solution contained 475 cc. of the former and none of the latter. The initial pH values were 6.55 and 6.45 for the low- and high-calcium solutions, respectively.

TABLE V

THE TOXICITY OF 500 P.P.M. OF STRONTIUM TO WHEAT IN RELATION TO THE CONCENTRATION OF CALCIUM IN THE NUTRIENT SOLUTION

SALT ADDED TO NUTRIENT SOLUTION	TRIPPLICATE WEIGHTS, EACH OF THREE PLANTS, WITH THE FOLLOWING CONCENTRATIONS OF CALCIUM		
	50 P.P.M.	275 P.P.M.	500 P.P.M.
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
None (controls) .....	4.43	4.91	3.04
	4.08	4.27	3.60
	3.96	4.07	3.51
Average .....	4.16	4.42	3.38
Strontium chloride .....	2.19	2.94	3.38
	2.16	3.24	3.86
	2.50	4.00	3.12
Average .....	2.28	3.39	3.45
Strontium nitrate .....	2.28	3.95	3.45
	1.90	3.94	3.54
	2.29	3.10	3.87
Average .....	2.16	3.66	3.62
Potassium chloride .....	3.82	3.93	3.98
	4.32	4.09	3.53
	4.50	4.63	3.23
Average .....	4.21	4.22	3.58
Potassium nitrate .....	4.80	4.14	2.66
	4.53	4.45	3.78
	4.64	4.40	3.59
Average .....	4.66	4.33	3.34

The toxicity of barium also is inhibited by calcium (12, 28, 29, 30, 37), which would seem consistent with the present thesis. However, calcium seems to be such a general antagonist (29) as to render uncertain the significance of its effects on its relatives, magnesium, strontium, and barium.

#### MISCELLANEOUS EXPERIMENTS

The question then arose as to whether phosphorus, potassium, and especially calcium were specifically antagonistic for arsenic, rubidium, and strontium, respectively, as assumed by the hypothesis which led to their selection for study, or whether their inhibiting effects might be more general. Accordingly arsenic toxicity was tested in the graded potassium and calcium solutions, rubidium toxicity with reference to phosphate and calcium levels, and strontium with reference to potassium. Only negative results were obtained, which seemed to corroborate the assumption of specificity in the antagonistic effects obtained with the chemically related pairs. However, McCool obtained positive effects of potassium on strontium toxicity (29).

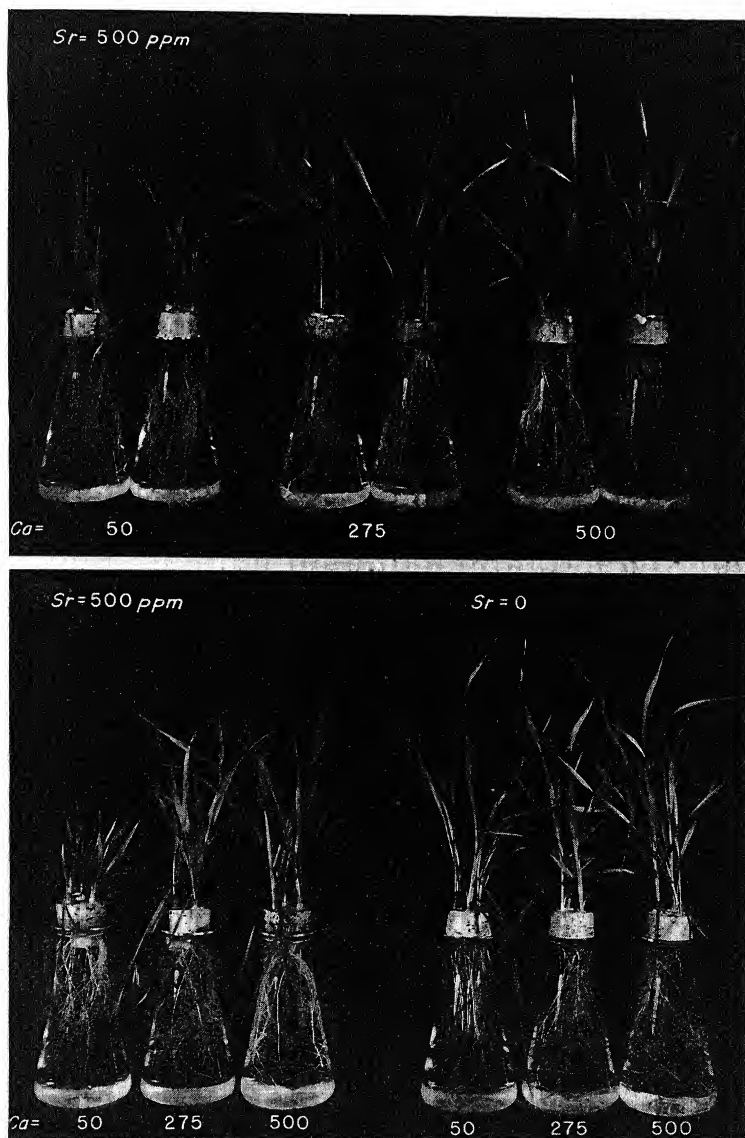


FIG. 5. Upper row: Effect of calcium (50, 275 and 500 p.p.m.) on the toxicity of strontium chloride (500 p.p.m. of Sr) to wheat in duplicate cultures. Lower row: Effect of calcium (50, 275 and 500 p.p.m.) on the toxicity of strontium nitrate (500 p.p.m. of Sr) to wheat. Controls without strontium to the right.

### Discussion

The basic concept of what may be termed "mass antagonism" is simply that of a mass effect of an essential element on the proportionate intake, and utilization in organic syntheses, of a toxic element sufficiently similar chemically as to preclude any considerable selectivity. Large effects can be expected only with the major essential elements, which are absorbable in relatively large quantities without injury to the plant. The total intake of the two related elements, the one essential, the other toxic, is presumably determined by the gradient established by the plant's metabolism of the essential one; and the intake of the toxic element decreases, in consequence, with increasing availability of the non-toxic one. This relation has been shown by chemical analyses of plants to obtain between selenates and sulphates (17, 19, 21), and the assumption that a similar situation might obtain with respect to the salts of analogous pairs of elements initiated the present investigations.

The literature dealing with the mineral nutrition of plants describes many instances of the interdependence of absorption rates of the elements, and of various antagonistic effects, antagonism being defined as the hindrance of one salt on the absorption and toxicity of another. The researches of LOEB with animals and of LOEW and OSTERHOUT with plants are outstanding in this field. For summaries of some of the many contributions to the subject, reference is made to reviews already published (11, 29, 35).

Of these various instances of antagonism, an outstanding one is that of calcium and magnesium, which seems likely to be of the type described in the present paper. These two elements are adjacent to each other in division A of group II of the periodic table. LOEW (28) postulated harmful substitution of magnesium for calcium in a calcium-protein compound of the nucleus and chloroplast, with the protective action of excess calcium a simple mass effect. Similarly LOEB (27) assumed, in explanation of physiologically balanced solutions, that cations enter into combination with proteins and can be replaced by other kinds of cations from the external solution "in accordance with the law of the mass." KEARNEY and CAMERON (25) concluded that "the theory that ions furnished by the dissociation of electrolytes form intimate combinations with the proteids of protoplasm, and that their mutually antagonistic effect expresses itself in a replacement of one kind of ion by another as a result of change in the composition of the surrounding solution, would appear to afford the key to this problem," that is, the problem of "the effect of one kind of ion in counteracting the physiological action of another kind." MEYER's finding (31) that the relative rather than the absolute amounts of lime and magnesium available determine toxicity by virtue of absorption into the plant in the same ratio as that in which they occur in the soil is consistent with the idea.

The possibility of substitution of chemically related ions in plant metabolism has been rather thoroughly explored by early investigators, both with respect to selenium and sulphur, as referred to elsewhere (18), and the other three pairs of elements dealt with in the present paper.

JADIN and ASTRUC (24) assumed that arsenic forms compounds in plant cells analogous to those formed by phosphorus. They refer to earlier work by GAUTIER and BERTRAND as establishing the fact of such substitution of arsenic for phosphorus. BOUILHAC (5) and STOKLASA (36) concluded that arsenates may partially replace phosphates in nutrition because, in the absence of sufficient phosphate, growth is improved when some arsenate is present. The extreme toxicity of arsenic to protoplasm and the very slight capacity of plants to accumulate it in the leaves (6) seem to indicate that arsenic injury to the tops is mostly a secondary effect of injury to the roots (where analyses have shown most of the absorbed arsenic accumulates (32, 39) rather than the result of substitution of arsenic for phosphorus in synthetic processes in leaves.

With respect to rubidium and potassium there are reports by ALTEN and GOTTWICK (1), LOEW (28), BLANCK *et al.* (4), SCHARRER and SCHROPP (33), HELLER *et al.* (15), and ARNDT (2). The toxicity of rubidium was interpreted as evidence of its non-substitutability for potassium in nutrition. But incidentally the fact was noted by some (2, 15) that a high potassium content was associated with reduced rubidium intake and reduced injury. ARNDT (2) showed that rubidium toxicity is a function of the rubidium/potassium ratio, a 1:3 ratio rendering the rubidium non-toxic. These reports showed that rubidium does not replace potassium harmlessly. The observations of antagonism of rubidium injury by potassium can be interpreted as the mass effect of the latter on the chance of harmful substitution of rubidium in the synthesis of compounds normally formed with potassium.

A similar situation with respect to a strontium-calcium relationship was revealed by a search of the literature. The claim (14) that calcium could be replaced by strontium in the nutrition of the plant was questioned on the grounds of the toxicity of strontium; but incidentally the discovery was made that strontium injury could be reduced or prevented by increasing the available calcium above a certain level (12, 28, 29, 30, 34, 37), a fact in accord with the assumption of harmful substitution of strontium for calcium.

If harmful replacement of a nutritive element by a toxic element occurs, an excess of the former would be expected on theoretical grounds to reduce the symptoms produced by the toxic element by reducing the chances of the replacement occurring. That this consequence of substitution was not stated by the earlier investigators is probably due to the fact that harmless utilization was being sought, and the possibilities and implications of harmful replacement were not followed up. Their work demonstrated that the essential

elements are not functionally or physiologically replaceable, but they did not demonstrate chemical irreplaceability nor preclude the possibility that the plant injury observed was caused, in part at least, by such substitution.

There are several reports of decreased absorption of the essential element of these pairs in the presence of the more toxic one, as should be the case if the total were established by the plant's metabolism of the essential element, and if the proportionate intake of each depends on their proportionate availability. Thus MEYER (31) noted that fertilizing with magnesium reduced the calcium while increasing the magnesium content of the plant; HASELHOFF (14) noted that increasing the strontium in the nutrient solution decreased the calcium while increasing the strontium intake; and others (1, 15) found that increasing rubidium absorption was associated with decreasing potassium intake.

Analyses of plants of different species have shown direct correlations between absorption of the elements of some of these pairs. Selenium absorption has been found to parallel that of sulphur (20), potassium that of rubidium (9), and strontium that of calcium (9), suggesting that, by virtue of their chemical similarity, the intake of the toxic element of each pair is quantitatively determined by the particular species' requirement of the essential one. Significant also is the work of COLIN and LAVISON (8) showing some differences but also certain significant similarities in the absorption and translocation of strontium and calcium.

A survey of the literature on permeability indicates that the rates of penetration of chemically similar ions, such for instance as sodium and potassium, or rubidium and potassium, are not equal (7). The physical properties of ions and of the root membranes can reasonably be assumed to be factors in the physical aspects of penetration, resulting in inequality of absorption regardless of the degree of chemical relationship. The concept of antagonism proposed to explain the phenomena described in the present paper is based on the idea that the root membrane has no capacity for selectivity beyond that inherent in such physical properties. The dominant factor is seen to be the physiological demand of the plant for a particular element, transmitted to the root through what may be termed the metabolism gradient for that element. The chemical nature of the available elements would seem to determine their response to this gradient. Incapacity to discriminate between the closely related ones is assumed to result in indiscriminate utilization and consequent damage to the plant in proportion to the relative rather than absolute availability of the toxic element.

### Summary

1. Arsenic injury is a function of the available phosphate concentration, with the protective arsenic/phosphorus ratio in nutrient solutions near 1:5.

2. Rubidium injury is a function of the available potassium concentration, with the protective rubidium/potassium ratio in nutrient solutions near 1:2.

3. Strontium injury is a function of the available calcium concentration, with the protective strontium/calcium ratio in nutrient solutions near 1:1.

4. As a working hypothesis to explain these effects, the assumption is made that they are the result of some degree of unselectivity in absorption and utilization of chemically related elements, with the result that when an organic molecule is synthesized, the chance of harmful substitution of the toxic element for the essential nutrient depends on the proportionate availability of the two. If the assumption proves to be correct, this particular type of antagonism may appropriately be designated "mass antagonism."

CEREAL CROPS AND DISEASES

BUREAU OF PLANT INDUSTRY

U. S. DEPARTMENT OF AGRICULTURE

WASHINGTON, D. C.

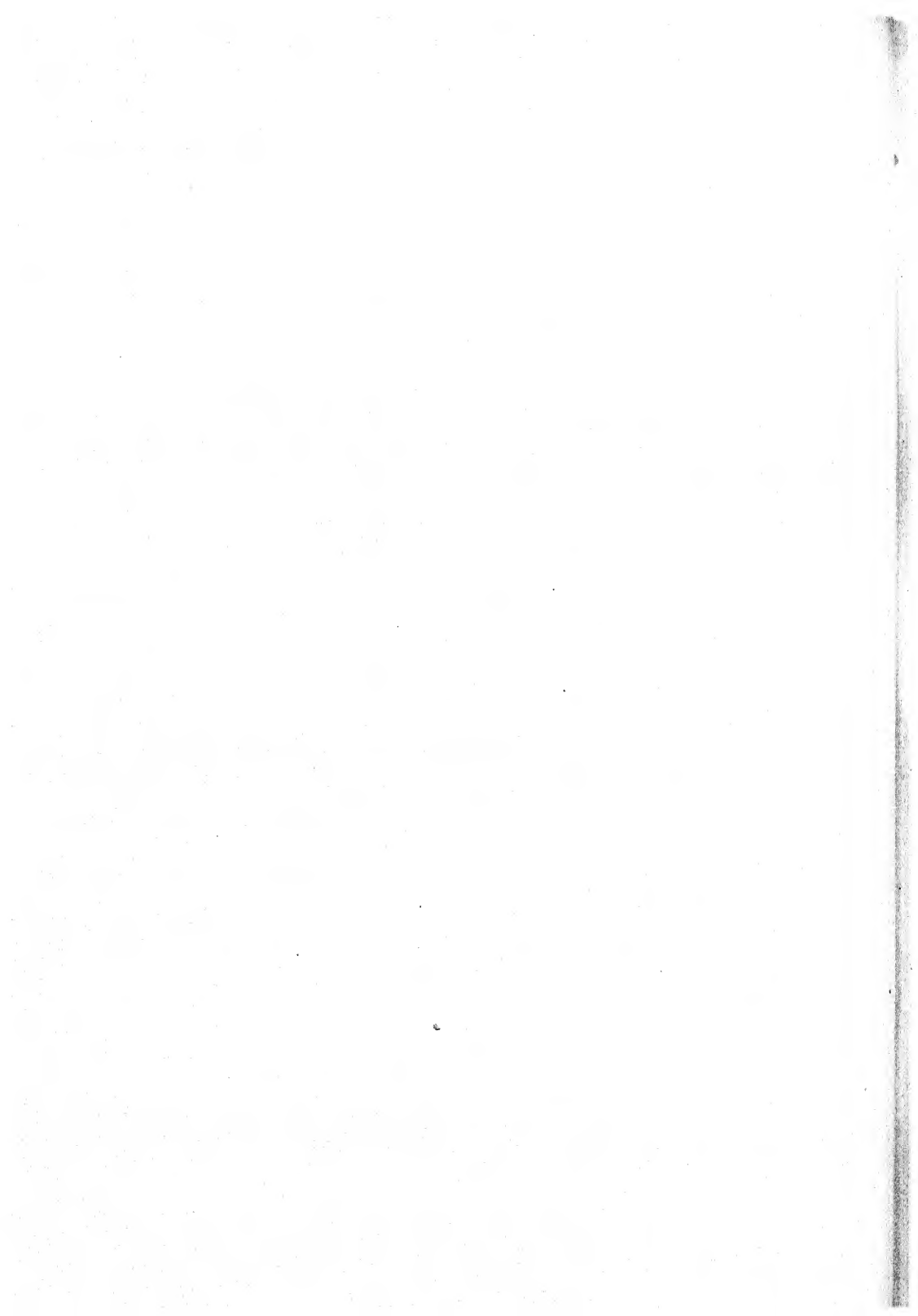
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# RÔLE OF NITROGEN IN FUNGOUS THERMOGENESIS<sup>1</sup>

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(WITH TWELVE FIGURES)

## Introduction

Thermogenesis by fungi has been definitely proved by MIEHE (10, 11), JAMES (7, 8), JAMES, RETTGER and THOM (9), NORMAN (12), GILMAN and BARRON (3) and others. The relation of substrate-composition to the thermogenic activity of the fungi involved has, however, received but little attention. Although the data presented by HARRISON (4) and those by NORMAN (13) clearly indicated the dependence of this process on the carbohydrate content of the substrate; the relationship that the nitrogen content, both as to type and amount, had to this process had not been investigated.

Various investigators have recognized that nitrogen added to substrates high in cellulose, hemicellulose, and similar plant products, bears an important relation to decomposition of these materials by fungi. BARTHEL and BENGTSSON (1) found that the favorable influence of stable manure upon cellulose fermentation in the soil was exerted by the nitrogen added with the manure. The soil micro-flora in this case was not limited to fungi. WAKSMAN (17) also found that nitrogen additions greatly increased the cellulose-decomposing activity of the soil micro-flora, except under anaerobic conditions. WAKSMAN and DIEHM (18), working with pure cultures of various fungi growing in a sand substrate containing additions of hemicellulose compounds and nitrogen, found that the ratio of nitrogen assimilated to hemicellulose decomposed was 1:35.5 for mannan, 1:16.7 for xylan, and approximately 1:35 for galactan. Further, in an experiment with fungous cultures growing in a liquid medium composed of ground corncobs with additional nitrogen, these investigators found that the ratio of nitrogen assimilated to corncob-xylan decomposed was 1:34.5. NORMAN (13) grew a number of fungi on oat straw with added ammonium carbonate and found that the "nitrogen factor" ranged from 0.50 for a species of *Phoma* to 0.83 for *Aspergillus terreus* Thom. He found, further, that the "nitrogen equivalent" was 3.33 for *A. terreus*—about an average for the fungi studied. This figure corresponds to a ratio of 1 part nitrogen to 30 parts organic matter and is quite similar to that determined by WAKSMAN and DIEHM for hemicellulose.

Although considerable work has been done on the relation of nitrogen to fungous decomposition of cellulose and related materials, comparatively few

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investigations have been made with reference to its rôle in thermogenesis. JAMES, RETTGER and THOM (9) found that, in the process of microbial heating in unsterilized cracked corn, total nitrogen remained practically constant, but that ammonia nitrogen very markedly increased, indicating a considerable transformation of organic nitrogen to the ammonia form, which, however, was not removed as such from the flask. NORMAN (12) working on thermogenesis of several fungi in cultures of oat straw, added nitrogen to the substrate in the form of ammonium nitrate, but did not test the influence of different amounts of nitrogen.

The problem was investigated in two of its aspects: first, the influence of various kinds and amounts of nitrogen upon temperatures attained in insulated cultures; and second, the influence of these compounds upon the general activity of the fungi, as measured by the loss in dry weight effected by them in substrates of similar composition.

### Material and methods

#### CULTURES AND SUBSTRATE EMPLOYED IN THE EXPERIMENTS

One culture each of *Aspergillus flavus* Link, *A. terreus* Thom, *Penicillium oxalicum* Currie and Thom, and *Rhizopus tritici* Saito was obtained from J. W. HARRISON of the Botany Department of Iowa State College in September, 1931. These fungi had been isolated from alfalfa hay in the summer of 1930, and were chosen because of their thermogenic capacity (4).

In order to study the effect of nitrogen upon the thermogenic activity of these organisms, a substrate low in nitrogen was required, yet one suitable in other respects for their rapid development, and sufficiently porous to permit aeration in thermos-flask cultures. Corncob meal was found to be satisfactory. Well-dried corncobs of the 1930 crop were obtained from the Agronomy Farm of the Iowa Agricultural Experiment Station and ground in a Wiley mill to pass a sieve with 2-mm. apertures. The meal was then carefully mixed and stored in earthenware jars in the laboratory.

Corncobs are composed primarily of cellulose, pentosans, and lignin. Representative samples of the meal, prepared as described above, were analyzed for moisture, total nitrogen and ash content, with the following results:

Moisture (varying during the year)	7.10-9.43 per cent. of total weight
Total nitrogen (Kjeldahl method) ...	0.40 per cent. of dry weight
Ash .....	1.24 per cent. of dry weight

Moisture content is expressed as per cent. of the total weight, but because of the variation in moisture during the year and since all figures relating to nitrogen in the following experiments are referred to moisture-free meal, the percentages of the other two components are based on dry weight. The

percentage of total nitrogen, as here determined, corresponds rather closely to that given by HENRY and MORRISON (5) as an average figure. They state that elemental nitrogen comprises approximately 16 per cent. of crude protein, and thus the figure which they give is equivalent to 0.36 per cent. total nitrogen (on the dry weight basis), as compared with 0.40 per cent. obtained from the meal used in these experiments.

For the experiments with thermogenesis, 70-gm. samples of corncob meal were placed in 500-ml. Erlenmeyer flasks, which were plugged with cotton and sterilized by autoclaving one hour at 15 pounds pressure on each of four consecutive days. The samples were then poured into one-pint thermos flasks which had been autoclaved one and one-fourth hours at 15 pounds. For experiments with loss in dry weight, 5-gm. samples were placed in 50-ml. Erlenmeyer flasks, which were plugged with cotton, and autoclaved 30 minutes at 15 pounds on each of four consecutive days.

To provide the desired moisture content and to add the proper amount of nutrients, solutions of the necessary ingredients were prepared, placed in containers of convenient size, and autoclaved 25 minutes at 15 pounds. These were later mixed into the samples of meal. In the thermogenesis experiments, however, this step was delayed, as described later in connection with the inoculation process. The moisture content was brought to approximately 70 per cent. in all cases, since this percentage of moisture had been found in preliminary trials to be satisfactory for both the thermogenesis and the loss-in-dry-weight experiments.

#### INOCULATION AND GROWTH OF THE CORNCOB-MEAL CULTURES

To prepare inoculum, cultures of the organisms were grown at laboratory temperature on potato-dextrose-agar slants for a sufficient length of time to permit abundant sporulation. In the thermogenesis experiments, inoculation was performed in the following manner: single tubes of inoculum were used for each sample of meal, spore suspensions were prepared in the nutrient solutions mentioned above and poured into the thermos flasks containing the sterile meal. The flasks were temporarily closed with rubber stoppers, which had been washed in 50-per cent. alcohol, and were thoroughly shaken to mix the spores and medium and to provide for better aeration. The flasks were allowed to remain in a horizontal position for approximately one-half hour, and were rotated from time to time to facilitate absorption of the solution. Each flask was then fitted with a cotton plug, a thermometer, and an aeration tube consisting of a section of capillary glass tubing of suitable length, with the lower tip completely recurved to prevent stoppage by particles of meal. Both the thermometer and the aeration tube were washed by immersion in 50-per cent. alcohol before insertion into the flask. The bulb of the thermometer and the curved end of the aeration tube were lowered to

a point near the bottom of the flask. Duplicate platings were made from each culture at the close of all experiments to demonstrate the presence or absence of contaminants.

Since each loss-in-dry-weight experiment required the inoculation of a relatively large number of samples, the needle method was employed in preference to the use of spore suspensions. After the nutrient solutions had been introduced into the samples of sterile cob meal, the flasks were shaken sufficiently to loosen the meal and to facilitate aeration; a mass of spores and hyphae of convenient size was then introduced into the center of the substrate by a single stab. All cultures for loss-in-dry-weight determinations were made in duplicate.

The term "check" was used in the results from all experiments to indicate comparable uninoculated samples. A single check was used in each thermogenesis test, except where otherwise indicated in the results; in the experiments on loss in dry weight all checks were in duplicate.

After inoculation, the thermos flasks were placed on a laboratory table and subjected to the influence of varying room temperatures; however, since all cultures of any given experiment were handled at the same time, the results obtained within each experiment are comparable. In addition to the aeration by diffusion through the cotton plugs, a slow current of air was drawn through the cultures for one-half hour each day by means of the vacuum pump. The rate of aeration was controlled by bubbling the air from each aeration tube through dilute sulphuric acid. The amount of air drawn through the cultures in this manner was approximately 1.8 liters per day. Temperatures were recorded from two to four times each day, depending upon the rapidity of the temperature change, and charts were prepared from these detailed data. The figures, however, indicate only significant points and general trends.

All cultures in the loss-in-dry-weight experiments, except in preliminary trials, were incubated at room temperature for a period of 28 days. Dry weights were determined by oven-drying at 100° C. for 48 hours, and the actual loss in dry weight was calculated by direct comparison with checks handled in a similar manner and oven-dried at the same time. Percentage loss in dry weight was computed on the basis of original dry weight of the meal in each culture.

### Experimental results

#### COMPARISON OF THE INFLUENCE OF ADDITIONS OF A SINGLE NITROGENOUS COMPOUND AND A FULL-NUTRIENT SOLUTION UPON THERMOGENESIS

In order to study the comparative effects of additions of a full-nutrient combination and of a single nitrogenous compound, alone, two nutrient solutions were prepared as follows:

Solution (1)		Solution (2)	
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	9.0 gm.	$\text{Ca}(\text{NO}_3)_2$	9.0 gm.
$\text{H}_2\text{O}$ (distilled) to make 1000.0 ml.		$\text{KH}_2\text{PO}_4$	3.0 gm.
		KCl	1.5 gm.
		$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.5 gm.
		$\text{FeSO}_4$	0.03 gm.
		Dextrose ( $\text{C}_6\text{H}_{12}\text{O}_6$ )	45.0 gm.
		$\text{H}_2\text{O}$ (distilled) to make 1000.0 ml.	

These solutions were sterilized by autoclaving in the usual manner. Two samples of meal received, respectively, 50-ml. lots of solution (1) and (2), together with sufficient sterile water to give the moisture content of 70 per cent. and were inoculated with *Aspergillus flavus*. Calcium nitrate was the only source of nitrogen in each solution, and the rate of addition was 0.08 gm. elemental nitrogen per 100 gm. dry meal.

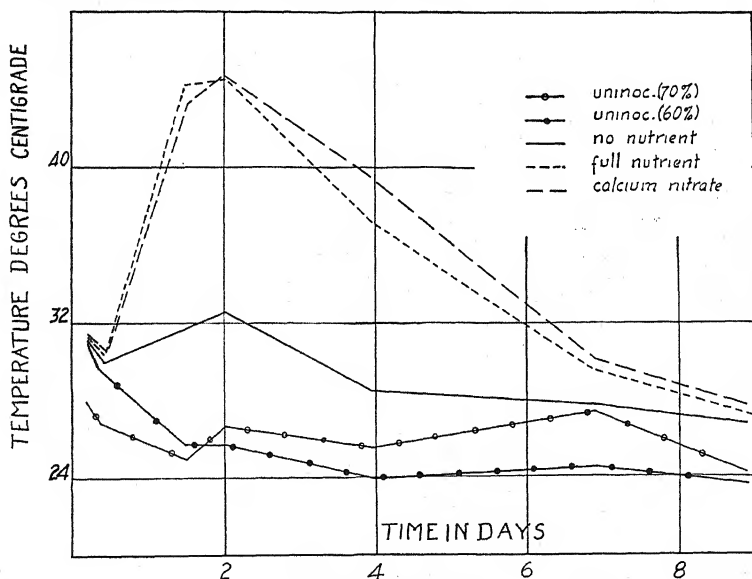


FIG. 1. Influence of different nutrient additions on temperatures in cornmeal cultures of *Aspergillus flavus*.

The results are presented in figure 1. The check, with moisture content equivalent to that in the two cultures, showed considerable fluctuation, which was found later to be caused by a faulty thermos flask. Another part of this experiment, dealing with the influence of moisture content upon thermogenesis, was being conducted at the same time; temperatures recorded for the check with 60 per cent. moisture are shown merely as indicative of the trend which temperatures in the 70 per cent. check normally would have taken. The results obtained from the inoculated cultures indicated that,



in this experiment, nitrogen, or at most calcium nitrate, alone, was the limiting factor in thermogenesis of *A. flavus*, since the addition of other nutrients resulted in but little difference in the temperatures reached. The culture containing the additional calcium nitrate without other nutrient additions reached its peak temperature of  $44.75^{\circ}\text{C}$ . at the end of the second day— $18.25^{\circ}$  above that of the check and  $12.25^{\circ}$  higher than the peak reached by the culture without added nutrient. The fact that nitrogen, and not calcium alone, was responsible for this pronounced stimulation of thermogenesis was demonstrated in subsequent experiments.

#### INFLUENCE OF VARYING ADDITIONS OF NITROGEN

When it was shown that the addition of a single nitrogenous compound to the meal greatly increased thermogenesis of *A. flavus*, experiments were made to test the effects of adding nitrogen in varying amounts. Three general types of nitrogenous compounds were separately employed, namely, organic-, ammonium- and nitrate-forms.

**THERMOGENESIS.**—Since the number of thermos flasks available was limited, only one organism, *A. flavus*, was used for experiments dealing with the influence of varying additions of nitrogen upon thermogenesis. Three experiments were conducted, in each of which the three compounds—asparagine, mono-ammonium phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ ), and calcium nitrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ )—were tested.

The results are presented in figure 2. Additions of asparagine ranged

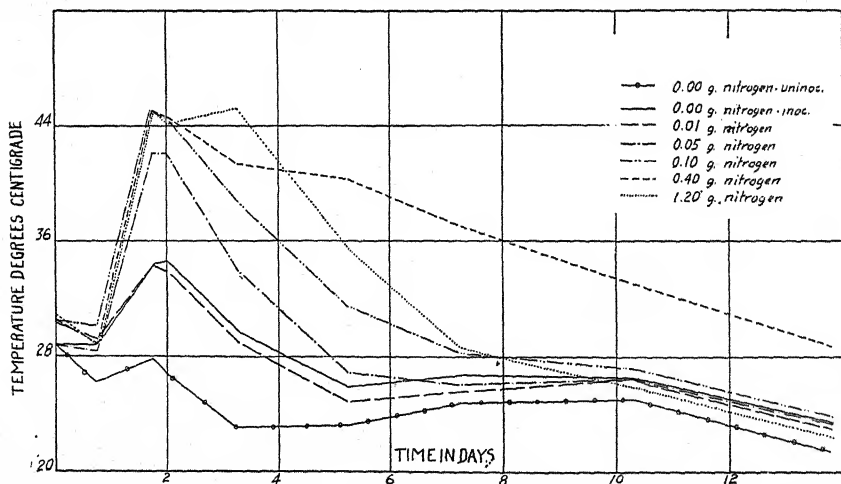


FIG. 2. Influence of varying additions of asparagine on temperature in cornmeal cultures of *Aspergillus flavus*.

from none to 1.20 gm. nitrogen per 100 gm. of dry meal, the upper limit being imposed by its low solubility. The pronounced increase in thermogenesis

with the additions of a single nitrogenous compound is again shown. All cultures containing asparagine additions of 0.10 gm. or more per 100 gm. of dry meal reached temperatures of 44.75° C. or higher—more than 10° above the peak reached by the culture without added asparagine and 17.5° or more above the check. Additions of asparagine in excess of 0.10 gm. nitrogen did not materially alter the rapidity of the response or the temperature peak, although a considerable prolongation of the period of heat production occurred in the culture containing the 0.40-gm. addition. The second peak and subsequent rapid fall in temperature of the culture receiving 1.20 gm. nitrogen may have been caused by a bacterial contaminant which was found at the close of the experiment. At this time the originally introduced organism seemed to be very largely destroyed, as evidenced by platings. Whether or not the contaminant could have had any appreciable influence upon the first peak in temperature is a matter of conjecture, but beginning with only a small amount of inoculum, it seems doubtful that it should have had any appreciable effect before the end of the second day.

The experiment with varying additions of mono-ammonium phosphate was similar to that in which asparagine was employed, except that additions of the nutrient were made over a much wider range—from none to 3.20 gm. nitrogen per 100 gm. of dry meal. The results are shown in figure 3. The differences observed between cultures receiving from 0.10 to 1.60 gm. nitrogen may be attributed to experimental error, such as that resulting from

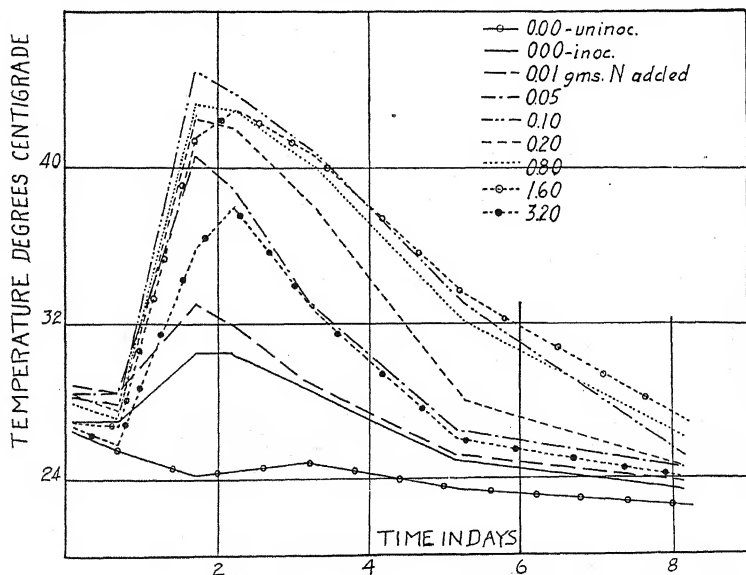


FIG. 3. Influence of varying additions of mono-ammonium phosphate on temperatures in cornmeal cultures of *Aspergillus flavus*.

slight differences in thermos flasks, in distribution of inoculum, and in conditions for aeration within the cultures. It is obvious, however, that 3.20 gm. nitrogen is well beyond the optimum. In this experiment, an addition of nitrogen as low as 0.01 gm. apparently resulted in a slight increase in thermogenesis, contrary to the experiment with asparagine.

In the third experiment of this series, varying additions of calcium nitrate were employed throughout the same range as with ammonium phosphate; and the results obtained (fig. 4) were quite similar. In these two experi-

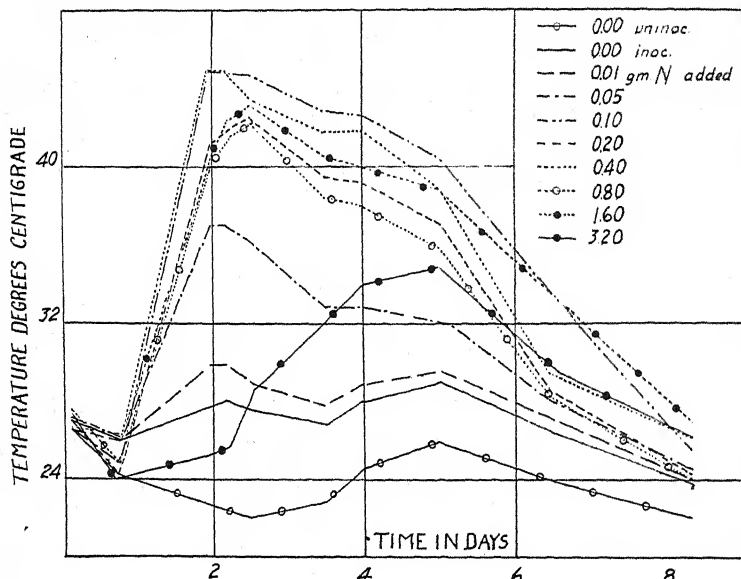


FIG. 4. Influence of varying additions of calcium nitrate on temperatures in corn-cob meal cultures of *Aspergillus flavus*.

ments, in which inorganic nitrogenous compounds were used, additions ranging from 0.10 to 1.60 gm. nitrogen per 100 gm. of meal produced only slightly differing results. Additions of 3.20 gm. and of 0.05 gm. nitrogen and below were decidedly less effective, although the amount of heating was markedly higher than where no nitrogen was added. With asparagine as with the inorganic compounds, additions ranging from 0.10 to 1.20 gm. nitrogen resulted in but slightly differing maximum temperatures. A decided prolongation of the period of heating occurred, however, in the one experiment where 0.40 gm. of nitrogen was added, as compared with 0.10 gm. The fact that the culture containing asparagine in the amount of 0.01 gm. nitrogen reached a maximum temperature slightly lower than the culture with no added nitrogen is probably not significant.

**LOSS IN DRY WEIGHT.**—In connection with those experiments in which varying nitrogen additions were tested for influence upon thermogenesis by

*A. flavus*, another series of experiments was conducted dealing with the effects upon fungous activity of varying additions of the same nitrogenous compounds, as shown by loss in dry weight. In these experiments relatively large numbers of cultures could be handled at one time, and thus all four of the fungi were employed in each test.

Before beginning with this series, however, a single experiment was carried out with *A. flavus* to determine the length of the growth period required for satisfactory comparisons in dry-weight loss. Four series of cultures—16 in each series—to which ammonium carbonate had been added at the respective estimated rates of 0.17, 0.22, 0.28, and 0.35 gm. of nitrogen per 100 gm. dry meal, and one series receiving no added nitrogen, were grown in comparison with uninoculated checks. Because of loss of ammonium carbonate in autoclaving, the amounts of nitrogen added to the cultures in the different series were estimated from total-nitrogen analyses made on ammonium-carbonate solutions prepared and autoclaved in exactly the same manner as those added to the cob-meal samples. At weekly intervals for a period of eight weeks, moisture-content and dry-weight determinations were made for one pair of cultures from each of the five series. At the end of eight weeks the rate of dry-weight loss in each series had apparently become almost nil and no loss whatever had occurred in the checks. By the end of four weeks dry-weight loss in every case was considerably greater than half the total loss reached in eight weeks and the relative position of each series, with respect to dry-weight loss, was very nearly the same throughout the period from the end of the third to the end of the eighth week. It seemed safe to conclude that a four-weeks' growth period should be sufficient for similar comparative tests, and consequently this period of growth was used in all subsequent experiments dealing with loss in dry weight.<sup>2</sup>

The influence of varying additions of asparagine upon dry-weight-loss effected by the four fungi—*A. flavus*, *A. terreus*, *Penicillium oxalicum*, and *Rhizopus tritici*—is shown in figure 5. In this experiment, as with that pertaining to the effects of asparagine upon thermogenesis, the low solubility of asparagine prevented any addition of this nutrient in a quantity greater than 1.2 gm. of nitrogen per 100 gm. dry meal. In every case progressive increases in dry-weight loss with larger additions of asparagine, up to 0.8 or 1.2 gm. nitrogen per 100 gm. of meal, occurred.

Figure 6 shows the results obtained in an experiment to determine loss in dry weight of cultures supplied with varying additions of mono-ammonium phosphate. The test was similar to that dealing with the effect of aspara-

<sup>2</sup> The standard error of a mean of two determinations,  $\pm 0.3002$ , calculated on the basis of ten cultures of *A. flavus* grown under the conditions of these experiments showed that the inoculating technique employed was sufficiently accurate, considering the magnitude of dry-weight losses. It is also evident that, under similar conditions, only relatively small differences between pairs of cultures would be required to be significant.

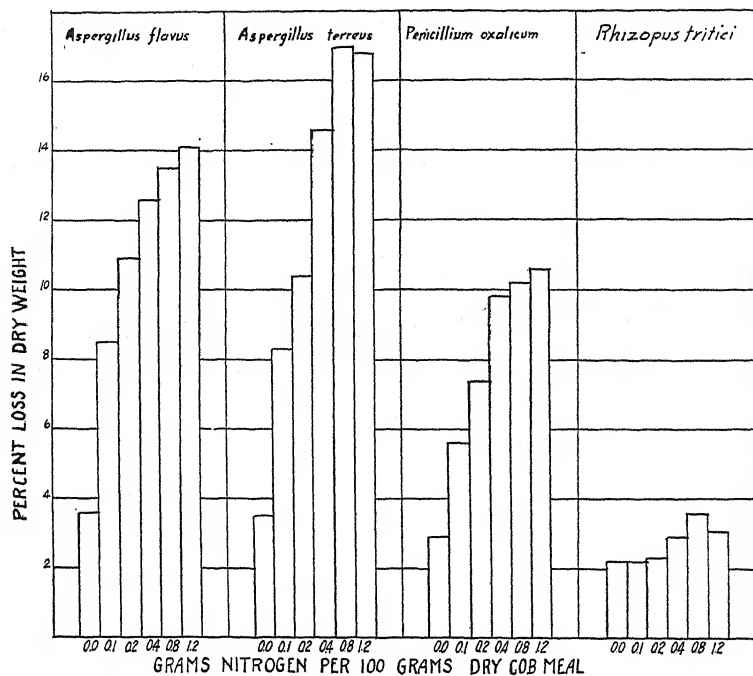


FIG. 5. Influence of varying additions of asparagine on loss in dry weight of corn-cob meal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

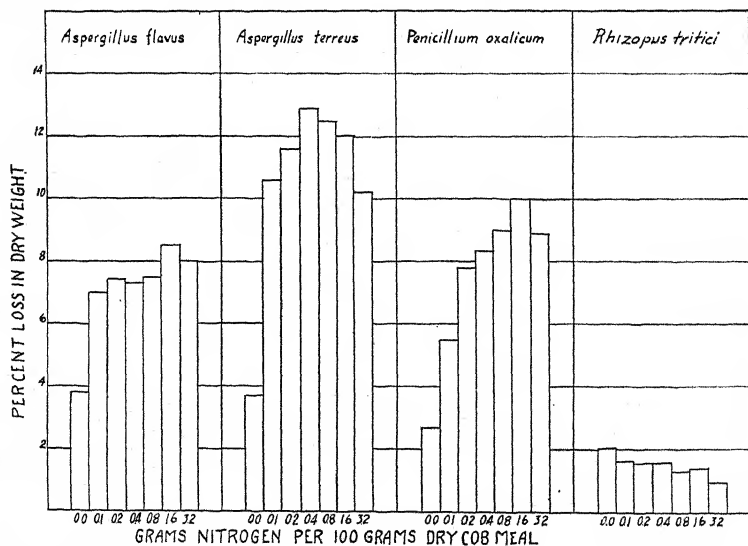


FIG. 6. Influence of varying additions of mono-ammonium phosphate on loss in dry weight of corn-cob meal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

gine, but additions of the nutrient covered a wider range—from none to 3.2 gm. nitrogen per 100 gm. of dry meal. The response of *Penicillium oxalicum* to ammonium phosphate was almost identical with its response to asparagine, and the optimum appeared to be near the same concentration of nitrogen. The two species of *Aspergillus* responded less vigorously to ammonium phosphate. With *A. flavus*, the optimum nitrogen addition, using the latter compound, appeared to approximate that for asparagine, but with *A. terreus* the optimum was apparently lower, about 0.4 or 0.4 to 0.8 gm. of nitrogen. With *Rhizopus tritici* a progressive decrease in dry-weight loss with the greater additions of nitrogen was found. This seems somewhat peculiar in view of the fact that with moderate additions of ammonium phosphate this fungus produced much more aerial mycelium in the early part of the growth period than where no nitrogen was added. The difference was still evident at the end of the period, though only very slightly. With the 3.2-gm. addition of nitrogen, no mycelial growth was apparent.

The effect of varying additions of calcium nitrate upon loss in dry weight is shown in figure 7. The experiment was similar to the preceding test with ammonium phosphate, except that additions of calcium nitrate ranged from none to 1.6 gm. nitrogen per 100 gm. of dry meal. As with ammonium phosphate, *R. tritici* showed decidedly negative results. In this case, however, the early mycelial growth, where only small amounts of nitrogen had been

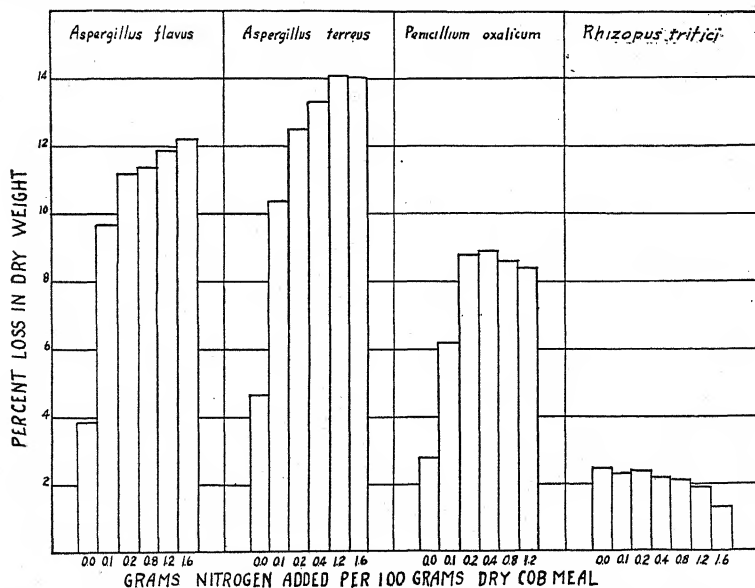


FIG. 7. Influence of varying additions of calcium nitrate on loss in dry weight of cornmeal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici* in 28 days.

added, showed but little, if any, increase in vigor, as compared with cultures with no added nitrogen; and, with the higher concentrations of nitrogen, growth was very weak throughout the experiment. *Aspergillus flavus* showed a response intermediate between that resulting from asparagine and from ammonium phosphate and here the optimum nitrogen addition was rather high, being about 1.6 gm. or above. *A. terreus* likewise showed an intermediate response, and the optimum nitrogen addition was approximately 1.2 to 1.6 gm. The response of *Penicillium oxalicum* was somewhat less than with either of the other two forms of nitrogen, and the optimum addition appeared to lie approximately between 0.2 and 0.8 gm. of nitrogen.

In comparing the effects of varying nitrogen additions upon thermogenesis and loss in dry weight of *Aspergillus flavus* cultures, it is notable that, whereas high additions of nitrogen—such as 1.2 to 1.6 gm. per 100 gm. of dry meal—gave greatest losses in dry weight where the fungus was allowed to grow for 28 days, considerably lower additions of nitrogen were sufficient to induce as rapid thermogenesis, during the peak of activity, as that obtained with larger quantities.

#### INFLUENCE OF DIFFERENT FORMS OF NITROGEN ADDED IN EQUAL QUANTITIES

In the work thus far reported only one form of nitrogen was used in each experiment. In order to compare the influence of different forms of nitrogen when added in equal quantities, two series of experiments were conducted, including four tests dealing with thermogenesis and one with loss in dry weight, in which all four organisms were employed. The following five compounds were compared:

- Asparagine
- Ammonium chloride ( $\text{NH}_4\text{Cl}$ )
- Mono-ammonium phosphate ( $\text{NH}_4\text{H}_2\text{PO}_4$ )
- Ammonium sulphate ( $(\text{NH}_4)_2\text{SO}_4$ )
- Calcium nitrate ( $\text{Ca}(\text{NO}_3)_2 \cdot 4 \text{H}_2\text{O}$ )

Each was used at the rate of 0.50 gm. of nitrogen per 100 gm. of dry meal.

**THERMOGENESIS EXPERIMENTS.**—In each of the four tests conducted relative to the influence of different forms of nitrogen upon thermogenesis, a different one of the four fungi was employed. Each experiment included one check and six cultures, one of which contained no added nitrogen. Each of the other five cultures was given an addition of one of the five compounds named above.

Figure 8 indicates the temperatures recorded in thermos-flask cultures of *A. flavus* throughout a period of 11 days. The striking feature of this experiment is the unusually high temperature of  $49.25^\circ \text{C}$ . reached by the

asparagine-containing cultures. Second to asparagine was calcium nitrate. The other cultures containing added nitrogen showed but slight differences, except for the ammonium-sulphate culture, which was noticeably lower. All

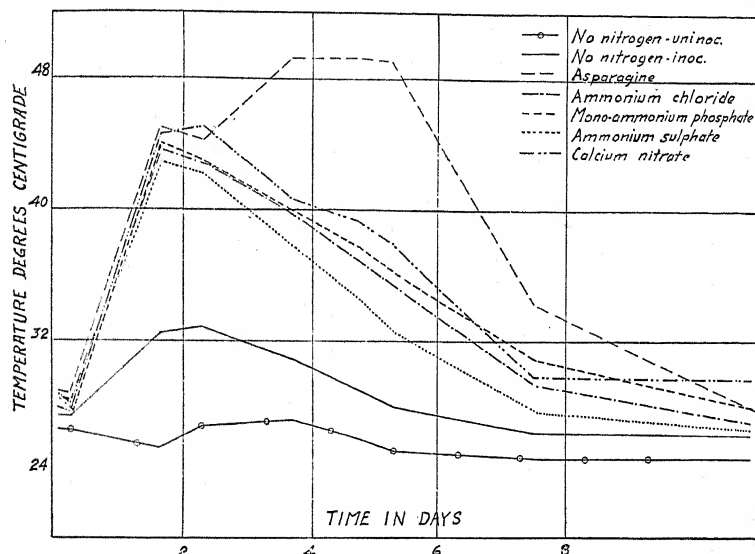


FIG. 8. Influence of additions of different forms of nitrogen on temperatures in corn cob meal cultures of *Aspergillus flavus*.

cultures supplied with nitrogen, in any form, showed temperatures far above those receiving no nitrogen addition.

The influence of different forms of nitrogen upon thermogenesis in cultures of *A. terreus* is shown in figure 9. Here, again, asparagine appeared to be superior to the other compounds, although the difference was much smaller than in the preceding experiment. The highest temperature re-

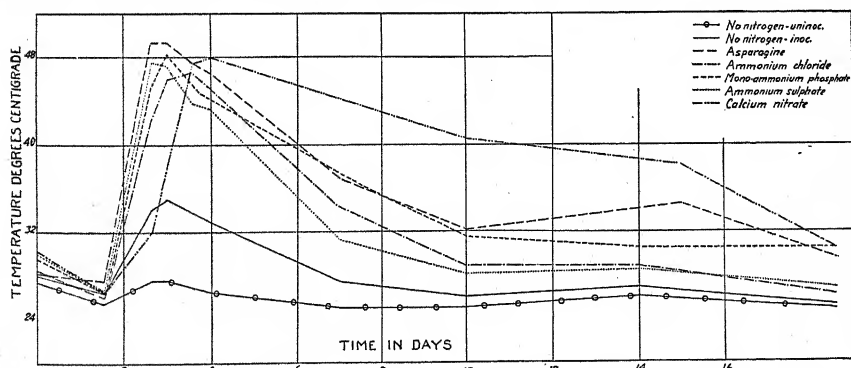


FIG. 9. Influence of additions of different forms of nitrogen on temperatures in corn cob meal cultures of *Aspergillus terreus*.



corded was  $49.25^{\circ}\text{C}$ ., which was held for a period of at least six hours. Calcium nitrate, as with *A. flavus*, seemed to rank second, although this culture did not quite reach the temperature attained by the ammonium phosphate culture. Somewhat lower in thermogenesis, but still far above the no-nitrogen culture, were those supplied with ammonium chloride and ammonium sulphate. In general, then, the response of *A. terreus* to additions of different forms of nitrogen was much the same as that of *A. flavus*.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Penicillium oxalicum* are presented in figure 10. As usual, asparagine produced the most rapid response, and the temperature reached was higher than in all other cultures except one. The striking feature of

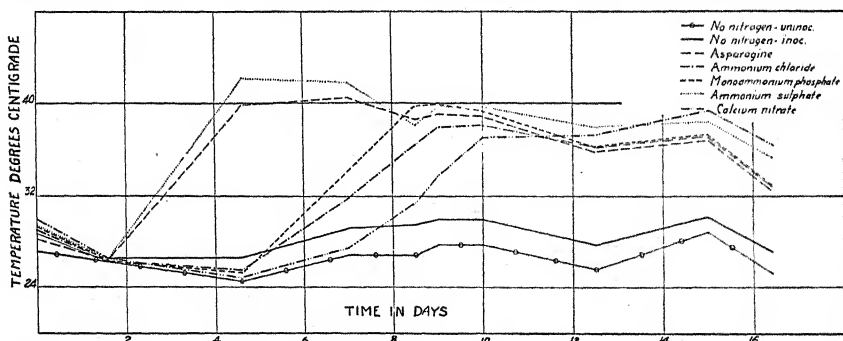


FIG. 10. Influence of additions of different forms of nitrogen on temperatures in cornmeal cultures of *Penicillium oxalicum*.

this experiment is the marked superiority of ammonium sulphate, which produced a temperature of  $42.25^{\circ}\text{C}$ .— $1.75^{\circ}$  higher than that attained by the asparagine culture. It should be pointed out here that the delay of other cultures in beginning thermogenesis may not be significant in this experiment, from the standpoint of nutritional value of the nitrogen compounds to the growing cultures, since the beginning of apparent growth of *P. oxalicum* in loss-in-dry-weight experiments was frequently delayed several days, and in some instances did not occur at all. In this experiment, consequently, thermogenesis in certain cultures may have been brought about by the effects of the added salts in decreasing spore germination, rather than by any toxic effect upon, or by unavailability to, the fungous mycelia. If it so happened that initial growth was largely inhibited in parts of any cultures, then a slow rise in temperature might be attributed to heating in only a part of the culture at any one time. In such cultures the temperature peak would necessarily have been somewhat lower, though a longer period of thermogenesis could have been expected. This may have been so with calcium nitrate, for example, as shown in figure 10. It seems probable that, if the suitability of

the several compounds from the start were considered, asparagine and ammonium sulphate were the most favorable for thermogenesis of *P. oxalicum* in this experiment. As in the two preceding experiments, all forms of nitrogen resulted in very pronounced increases in thermogenesis.

The effects of additions of different forms of nitrogen upon thermogenesis in cultures of *Rhizopus tritici* are given in figure 11. In this experiment, contaminants were found in four cultures at the end of the ninth day, which cast some doubt upon the validity of the results. In three of the cultures, however, those receiving ammonium-chloride, -phosphate, and -sulphate, re-

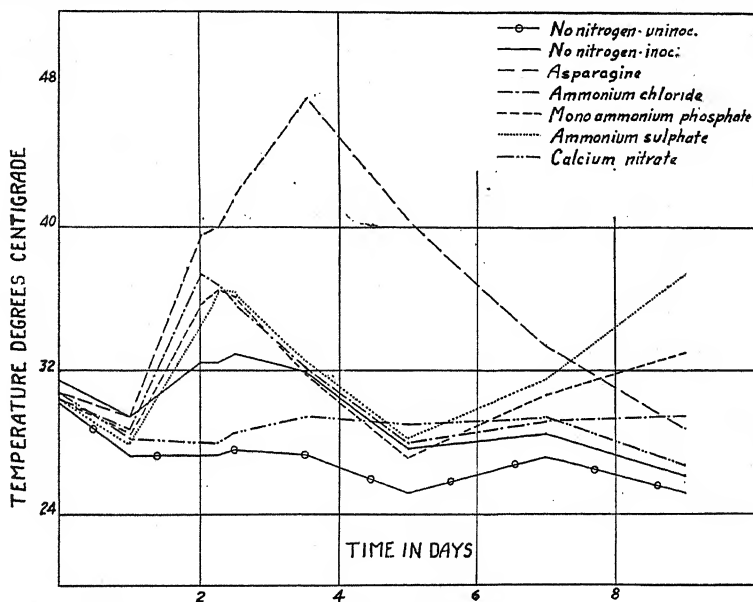


FIG. 11. Influence of additions of different forms of nitrogen on temperatures in cornmeal cultures of *Rhizopus tritici*.

spectively, it seemed quite probable that no appreciable effect could have resulted from the presence of the contaminants before the third or fourth day at the earliest. This assumption is based upon the fact that the contaminants were fungi and were apparent in only a part of each of the cultures; further, it appears that the period of secondary heating by these organisms began somewhere between the fifth and sixth days. It is a matter of conjecture whether or not the bacterial contaminant in the asparagine culture had any appreciable influence upon the peak temperature which was reached during the fourth day. It is doubtful, however, if its effect was of any importance during the period when the other cultures were at their peak temperatures—about the end of, or shortly after, the second day.

If it may be assumed that the effect of contaminants was absent or negligible until after the first two and one-half days, then asparagine was apparently the most suitable source of nitrogen, with ammonium chloride ranking next. The effect of calcium nitrate was decidedly inhibitory, since the maximum rise in temperature—above the check—was  $1.75^{\circ}$  less in the calcium nitrate culture than in the culture containing no added nitrogen. The latter reached a temperature of  $33.0^{\circ}$  C.— $5.25^{\circ}$  above the check. This is the only case, in this series of four experiments in which the addition of a nitrogenous compound failed to result in a temperature considerably higher than that reached in the comparable no-nitrogen culture. It may be mentioned that, in the next experiment (fig. 12), dealing with loss in dry weight of cultures of these four fungi and comparing the same five compounds, the only culture which did not respond to nitrogen by producing more abundant apparent mycelial growth during the early part of the 28-day period was *R. tritici*, supplied with calcium nitrate.

LOSS IN DRY WEIGHT.—As mentioned in the preceding paragraph, an experiment was conducted in which the influence, upon loss in dry weight, of additions of the five different forms of nitrogen previously tested was com-

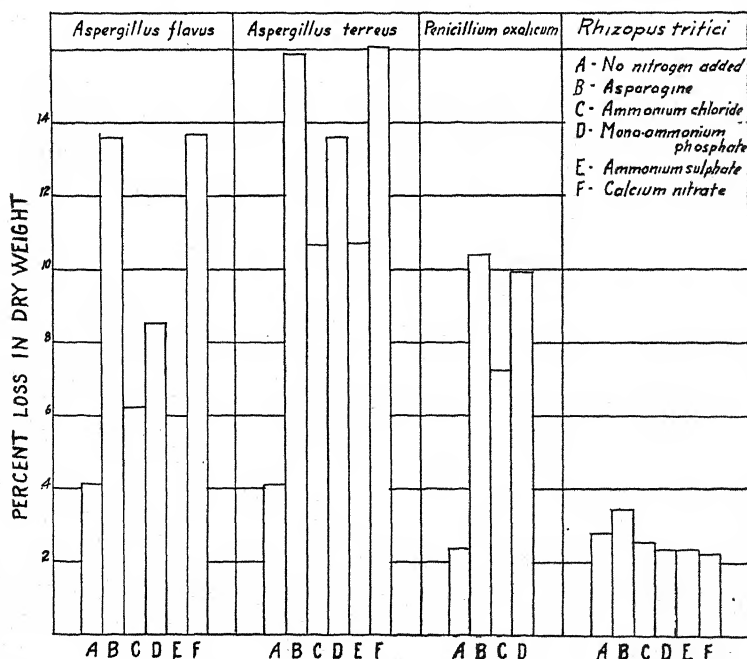


FIG. 12. Influence of additions of different forms of nitrogen on loss in dry weight of corn-cob meal cultures of *Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici*.

pared for the four fungi used in other experiments. The results are presented in figure 12.

The correlation, generally, between the results obtained in this and the four preceding experiments was noteworthy. For *Aspergillus flavus* and *A. terreus*, asparagine and calcium nitrate were distinctly superior to all other compounds used as sources of nitrogen, both for thermogenesis and loss-in-dry-weight cultures, and ammonium phosphate ranked next in both types of experiments.

*Penicillium oxalicum* produced no apparent growth in the samples of meal containing ammonium sulphate and calcium nitrate in the loss-in-dry-weight experiment (fig. 12). However, the distinct response to additions of asparagine and ammonium phosphate agreed with the results obtained in the thermogenesis experiment with this organism (fig. 10).

With *Rhizopus tritici*, the superiority of asparagine was quite distinct in both types of experiments. With the three ammonium salts, the effect upon dry-weight loss was inhibitory, whereas these compounds considerably increased thermogenesis. In the experiment with loss in dry weight, the apparent mycelial development was much greater during the early part of the 28-day growth period in cultures containing additions of these salts than in no-nitrogen cultures. At the end of the period, however, this difference had disappeared. It seems probable, then, that the presence of these compounds made possible more rapid development, as well as thermogenesis, during the first few days after inoculation, but that products resulting from their decomposition inhibited fungous activity after a longer period of growth, thus actually decreasing the total loss in dry weight in 28 days. Calcium nitrate resulted in the greatest reduction in dry-weight loss. As previously pointed out, it did not stimulate apparent mycelial growth even during the first part of the 28-day period, and correlated with this fact was its inhibiting effect upon thermogenesis.

### Discussion

From the data presented it is apparent that, under the conditions of the experiments, nitrogen was an important factor in limiting both thermogenesis and loss in dry weight in cultures of the four fungi studied. Table I summarizes the results obtained from additions of five different compounds, respectively, to the corncob-meal substrate.

Examination of this table shows a rather close correlation between thermogenesis and loss in dry weight. For both species of *Aspergillus*, asparagine and calcium nitrate were outstanding in producing the highest thermogenesis as well as the largest losses in dry weight; ammonium sulphate and ammonium chloride were least effective in both types of tests; and mono-ammonium phosphate was intermediate.

TABLE I

SUMMARY OF THE RESULTS ON THERMOGENESIS AND LOSS-IN-DRY-WEIGHT OBTAINED IN CULTURES OF FOUR FUNGI GROWING ON VARIOUS SUBSTRATES

FORM OF NITROGEN ADDED	MAXIMUM RISE IN TEMPERATURE AND LOSS IN DRY WEIGHT							
	<i>A. flavus</i>		<i>A. terreus</i>		<i>P. oxalicum</i>		<i>R. tritici</i>	
	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.	TEMP. RISE*	LOSS IN DRY WT.
	°C.	%	°C.	%	°C.	%	°C.	%
No nitrogen .....	7.00	4.01	7.25	4.01	3.00	2.38	5.25	2.82
Asparagine .....	23.50	13.59	21.75	15.91	15.25	10.39	19.75†	3.52
Ammonium chloride...	18.25	6.22	19.50	10.66	10.50	7.25	10.00	2.49
Mono-ammonium phosphate .....	18.50	8.55	20.50	13.64	13.00	9.96	9.00	2.38
Ammonium sulphate	17.50	5.95	20.00	10.72	17.75	.....	9.00	2.38
Calcium nitrate .....	19.00	13.69	21.50	16.07	11.75	.....	3.50	2.27

\* Influence of different forms of nitrogen on thermogenesis was tested in a separate experiment for each organism. The figures shown indicate maximum differences between checks and cultures.

† Maximum temperature may have been affected by a bacterial contaminant.

With *Penicillium oxalicum*, also, a correlation is shown by the partial data. The greatest amount of thermogenesis occurred in the culture containing ammonium sulphate, but unfortunately no loss-in-dry-weight data were obtained for this compound. Next in order in thermogenesis were the cultures containing additions of asparagine and mono-ammonium phosphate, respectively; and, as with the two species of *Aspergillus*, ammonium chloride was distinctly less effective. Likewise, among those loss-in-dry-weight cultures in which growth took place, asparagine gave the greatest response, mono-ammonium phosphate was second, and ammonium chloride third.

The results shown for *Rhizopus tritici* are of especial interest, in that only asparagine increased the loss in dry weight, whereas all forms of nitrogen used, except calcium nitrate, distinctly increased thermogenesis. An explanation of this seemingly peculiar occurrence has been proposed in connection with the presentation of the original data (figs. 11 and 12). It is sufficient to repeat here that all forms of nitrogen except the nitrate obviously stimulated early mycelial growth in the loss-in-dry-weight cultures, and it would seem that such a stimulation in the thermos-flask cultures was probably accompanied by more rapid decomposition of the substrate for a short period, and thus was responsible for the greater thermogenesis during this time. The accumulation of decomposition products of these compounds, however, was probably the cause for the decrease in the final loss in dry weight in 28 days—an effect not entering into the results appreciably during the short period in which the thermogenesis experiments were conducted. The dis-

tinued superiority of asparagine, as a source of nitrogen for *R. tritici*, is quite evident in its influence upon thermogenesis as well as upon loss in dry weight. As might be expected from the classification of molds by ROBBINS (15) calcium nitrate was not only unsuitable for this organism, but was decidedly inhibitory in its action. These results indicate an extension of the findings of ROBBINS (15) that *Aspergillus niger* has a higher apparent reducing intensity than *Rhizopus nigricans*. The data indicate that the same relation holds between *Rhizopus tritici* and *Aspergillus flavus*.

From the results set forth above it seems probable that the specific effect of each form of nitrogen in promoting thermogenesis depends directly upon the concomitant intensity of fungus decomposition of the substrate.

The influence of varying additions of three forms of nitrogen upon thermogenesis and loss in dry weight in cultures of *Aspergillus flavus* may be summarized as follows (table II).

TABLE II

THE INFLUENCE OF VARYING ADDITIONS OF THREE FORMS OF NITROGEN UPON THERMOGENESIS AND LOSS IN DRY WEIGHT IN CULTURES OF *Aspergillus flavus*

NITROGEN ADDED PER 100 GM. COB MEAL	MAXIMUM RISE IN TEMPERATURE AND LOSS IN DRY WEIGHT					
	ASPARAGINE*		MONO-AMMONIUM PHOSPHATE*		CALCIUM NITRATE*	
	TEMP. RISE†	LOSS IN DRY WT.	TEMP. RISE†	LOSS IN DRY WT.	TEMP. RISE†	LOSS IN DRY WT.
gm.	°C.	%	°C.	%	°C.	%
0.00	8.00	3.59	6.50	3.80	5.50	3.89
0.01	7.00	.....	8.75	.....	7.25	.....
0.05	15.00	.....	16.25	.....	14.50	.....
0.10	17.75	8.43	20.50	7.00	22.50	9.72
0.20	.....	10.87	18.25	7.38	20.50	11.18
0.40	18.75	12.61	.....	7.32	22.25	11.02
0.80	.....	13.54	19.25	7.54	20.25	11.39
1.20	17.50‡	14.13	.....	.....	.....	11.93
1.60	.....	.....	18.75	8.52	21.25	12.21
3.20	.....	.....	13.25	8.08	9.50	.....

\* Each form of nitrogen was tested in a separate experiment.

† Figures indicate maximum differences between checks and cultures.

‡ First peak in temperature is shown since second peak was probably affected by a bacterial contaminant.

In examining table II, the average maximum rise in temperature above the check for the no-nitrogen cultures was 6.67° C.; and for those cultures containing 0.01-, 0.05-, and 0.10-gm. additions of nitrogen it amounted to 7.67°, 15.25°, and 20.25° C., respectively. Above this point, increased additions gave results quite similar to that of 0.10 gm., except for the extremely large amount of 3.20 gm., which was obviously well beyond the optimum. With dry-weight loss, however, the results were somewhat different. With

each of the three forms of nitrogen, a progressive increase in percentage of loss in dry weight resulted from all the larger additions, except the extremely large amount of 3.20 gm.

Since the use of nitrogen in excess of 0.10 gm. per 100 gm. of meal did not materially increase thermogenesis—either in maximum temperature rise or in prolongation of heating—but did substantially increase loss in dry weight throughout a longer period (28 days), it might seem that small amounts of nitrogen were utilized by the fungus in the process of decomposition of the more readily available constituents of the corncob meal, during which time the most rapid development and respiration, together with greatest thermogenesis, took place. Following this period, with the aid of more nitrogen, the fungus was probably able to continue growth and respiration at a much less rapid rate, utilizing some of the more resistant carbohydrates present, and producing heat much more slowly. The more readily available food materials probably consisted principally of certain of the hemicelluloses, which comprise, according to BURKE (2) about 37 per cent. of corncobs, and the more resistant materials possibly included some of the true celluloses. This explanation is based largely upon the results of NORMAN (12), who found that the hemicelluloses were the constituents most rapidly lost in the early stages of decomposition of unsterilized oat straw, and who noted that this period corresponded with that of maximum thermogenesis in a vacuum flask containing similar material. The fact should not be overlooked that conditions for decomposition inside a mass of heating straw are decidedly different from those in straw which is not heating, though it seems doubtful whether this should greatly alter the sequence of decomposition.

The fact that the addition of a full nutrient combination was no more effective in stimulating thermogenesis of *A. flavus* than was an addition of the nitrogenous compound (calcium nitrate) alone, is of special interest, since it indicated that, so far as the food-nutrient composition of the substrate was concerned, nitrogen, or at most calcium nitrate, was the most important factor in thermogenesis in corncob-meal cultures of this fungus. Experiments in which other forms of nitrogen were employed demonstrated the fact that nitrogen, and not calcium, was the limiting factor.

### Summary

1. The influence of various nitrogen additions, and certain other factors, upon thermogenesis and loss in dry weight of corncob-meal cultures of four species of fungi was studied.

2. The organisms employed—*Aspergillus flavus*, *A. terreus*, *Penicillium oxalicum* and *Rhizopus tritici*—had previously been isolated from alfalfa hay, and found to be capable of considerable heat production in moist hay cultures.



3. Five forms of nitrogen—asparagine, ammonium chloride, mono-ammonium phosphate, ammonium sulphate and calcium nitrate—each greatly stimulated both thermogenesis and loss in dry weight when added to the substrate in cultures of *Aspergillus flavus*, *A. terreus*, and *Penicillium oxalicum*. With *Rhizopus tritici*, all forms of nitrogen markedly increased thermogenesis, with the exception of the nitrate form which showed a distinct inhibiting effect. Only asparagine increased dry-weight loss in cultures of this organism, all other compounds showing a depressing influence, with the nitrate being most effective in this regard.

4. Asparagine was found to be most generally suitable for the four fungi employed, both in its effect upon thermogenesis and upon loss in dry weight. The highest temperature recorded throughout the experiments—49.25° C.—was reached in cultures of *Aspergillus flavus* and *A. terreus*, each of which had been supplied with asparagine. The significance of these figures is shown by the fact that the maximum temperature above the check in both instances exceeded 21.50° C., as compared with cultures containing no added nitrogen, in which the temperatures reached were not more than 7.25° C. above the check with either fungus. The greatest loss in dry weight, in 28 days, occurring throughout the course of the experiments, was 16.95 per cent., which was found in cultures of *A. terreus* supplied with asparagine, and may be compared with 3.54 per cent. loss occurring at the same time in cultures of this organism containing no added nitrogen.

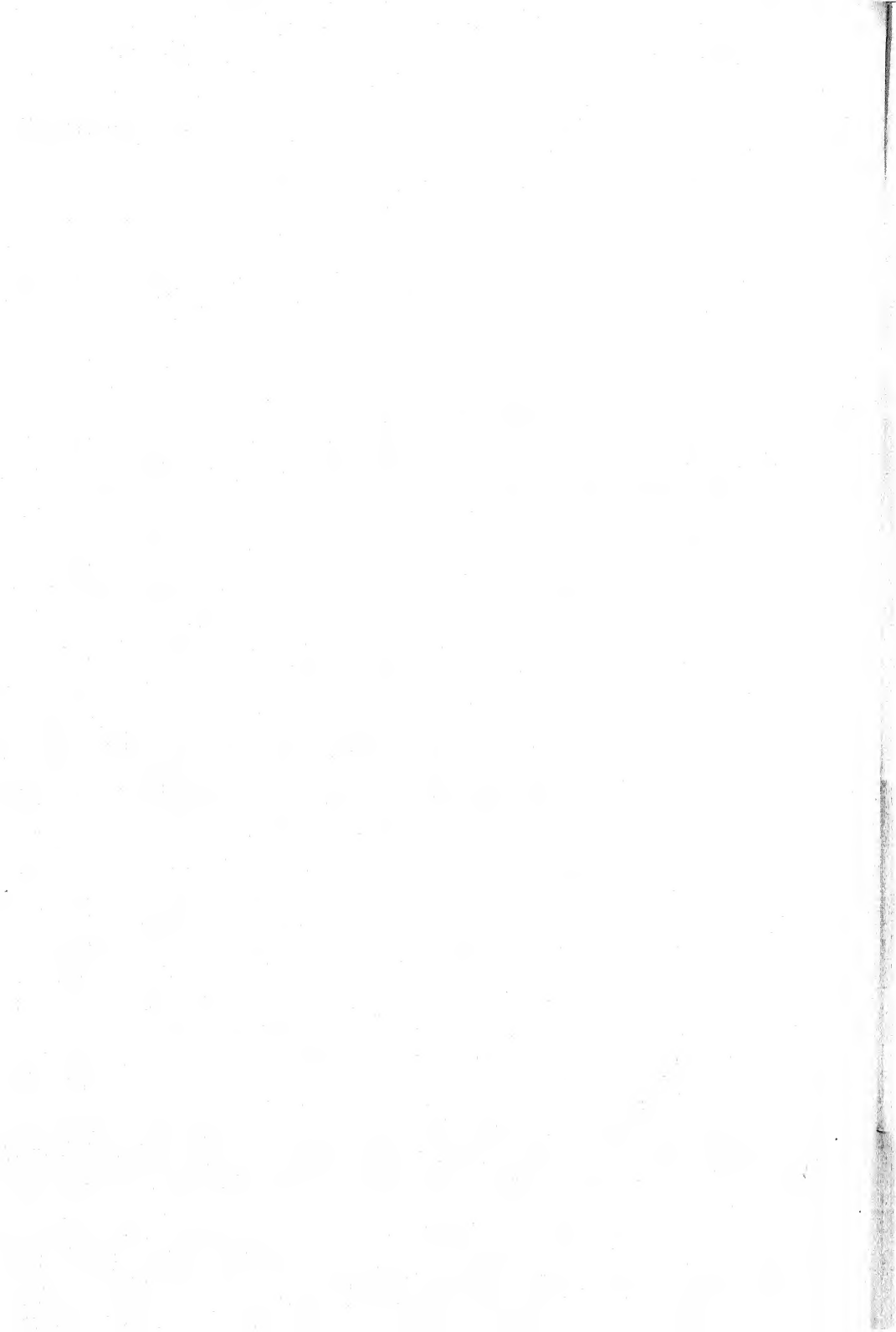
5. Nitrogen additions ranging from 0.01 to 0.10 gm. per 100 gm. of dry cob-meal generally resulted in progressive increases in thermogenesis in cultures of *A. flavus*. The average maximum rise in temperature above the check, for additions of three forms of nitrogen—asparagine, mono-ammonium phosphate, and calcium nitrate—at the rate of 0.00, 0.01, 0.05, and 0.10 gm. per 100 gm. of meal, were 6.67°, 7.67°, 15.25° and 20.25° C., respectively. Greater additions, ranging from 0.20 to 1.60 gm., inclusive, generally gave results similar to that shown for the 0.10-gm. addition, with an average of 19.64° maximum rise in temperature; the use of 3.20 gm. nitrogen, as mono-ammonium phosphate and calcium nitrate, resulted in an average maximum rise of only 11.38°.

6. Progressive increases in dry-weight loss in cultures of *A. flavus* followed the larger additions of asparagine, mono-ammonium phosphate and calcium nitrate, respectively, up to 1.2 and 1.6 gm. nitrogen per 100 gm. of dry cob-meal. The other three organisms differed considerably in response to various amounts of these compounds.

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# TRANSLOCATION OF CARBOHYDRATES IN THE SUGAR BEET<sup>1</sup>

O. A. LEONARD

(WITH EIGHT FIGURES)

## Introduction

This paper covers certain physiological phases of a series of investigations on the production of sugar beets in Iowa, and is intended to aid in the interpretation of pathological and cultural studies. In a plant whose commercial importance depends upon its sugar content, problems of photosynthesis, translocation, transformation, and storage obviously are important. The relation of the four principal carbohydrates of the sugar beet, sucrose, fructose, glucose, and dextrin to these processes has been the object of this investigation.

## Literature review

Literature dealing with the sugars in the leaves and roots of the sugar beet is voluminous, but only a small fraction of the work is related to photosynthesis, translocation, transformation, and storage. In this review, only that sugar beet work which has a particular bearing on the present problem will be referred to and it will be discussed in chronological order.

GIRARD (9) in his studies on the chemical composition of leaves and roots of sugar beets, found that sucrose in leaves fluctuated more between day and night than did the reducing sugars. At evening sucrose concentration was sometimes observed to be twice as great as it was the next morning, while reducing sugars sometimes remained constant. He concluded that sucrose is the first product of photosynthesis and is moved to the root as such. Both midribs and blades, however, were analyzed together. The high percentage of reducing sugars in the midribs undoubtedly masked diurnal variations which occurred in the blades. He was unable to detect reducing sugars in roots. MACQUENNE (13) contrary to GIRARD, believed that the reducing sugars were the first products of photosynthesis and that they migrated to the roots in that form, and were there condensed into sucrose.

LINET (11) investigated the proportion of glucose to fructose in different parts of the leaves. In the early stages of growth (July) glucose usually exceeded fructose in the blades, while in the petioles, fructose was found to be low in comparison to glucose. He believed that the excess of fructose in the leaves was due to glucose being consumed in the tissues by respiration more rapidly than fructose. Low fructose values for the petioles

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were believed to be due to fructose being used more easily than glucose in the formation of new tissues.

STRAKOSCH (21), employing microchemical methods, thought glucose to be the only sugar in the mesophyll cells of the sugar beet. Migration of glucose into the veins was followed by the appearance of fructose therein and later by the formation of sucrose. He believed that glucose was the first product of photosynthesis and that fructose was formed from it. In the veins and petioles, glucose and fructose were condensed to form sucrose and translocated to the roots in that form. PEKLO (16), also from microchemical data, concluded that the sieve tubes contained the greatest amount of sucrose.

ROBERTSON, IRVINE, and DOBSON (18) studied the distribution of enzymes in the blades, petioles, and roots of the sugar beet. They found that invertase was present in blades and petioles but was absent from the roots. They believed that invertase synthesized as well as hydrolyzed sucrose and, therefore, concluded that sugars were translocated to the root in the form of sucrose. STEPHANI (20) supported this view.

RUHLAND (19) held that, in the sugar beet, sugar migrates in the leaves not as sucrose, but as reducing sugars and perhaps mainly as fructose. Reducing sugars were considered to be condensed into sucrose upon entering the roots where they are stored and from whence they cannot be moved until drawn upon by growth. Reducing sugars at times actually decreased from the apex to the base of the petioles. Cells of the leaves were said to be permeable to fructose, glucose, sucrose, and more or less to all the sugars tested. Starch was formed in the leaves when any of these sugars was supplied externally.

CAMPBELL (4), working on the mangold plant which, like the sugar beet, is a variety of *Beta vulgaris* L., suggested that reducing sugars were the first carbohydrates to be formed as soon as daylight began. A little later the sucrose curve began to rise, and later still the "starch" curve. The sucrose curve did not appear to rise before the reducing sugars had reached their maximum, which they maintained throughout the period of illumination. Similarly, the "starch" did not seem to rise until sucrose had reached its maximum. Young leaves had a much higher level of reducing sugars than old leaves, but sucrose content was about the same. The fraction reported as "starch" was probably dextrin rather than starch.

In 1916, DAVIS, DAISH, and SAWYER (6) published the most complete work which had yet been done on *Beta vulgaris* L. Some of their conclusions were: (1) Starch was absent from the leaves, except in the very early stages of growth before the root had undergone appreciable enlargement. (2) Maltose was absent at all stages of growth. (3) In the midribs and petioles the hexoses were always greatly in excess of sucrose and varied greatly between

day and night and throughout the season, whereas sucrose remained relatively constant. The ratio of the hexoses to sucrose increased from the blade to the base of the petiole. As the season progressed, the predominance of the hexoses in the blades, midribs, and petioles became more marked. (4) Sucrose was assumed to be the first sugar of photosynthesis. It was hydrolyzed, they said, and moved to the root in the form of invert sugar. Once in the root it was again converted into sucrose and remained there until it was used for growth of the following year. (5) Sucrose is probably not synthesized by invertase.

TOTTINGHAM, *et al.* (22) have published data on the effects of climate on the sugar beet. They noted that temperatures of 30° C. limited photosynthesis. Fluctuations in reducing sugars and sucrose were observed during the day. Sucrose was frequently absent from the petiole, and less often from the blade. Reducing sugars were always present in both blade and petiole.

DOBY and HIBBARD (7) investigated the effect of nutrient ions upon enzyme activity in the sugar beet. They concluded that the quantity of enzyme is controlled by the type of nutrition, being greater in plants growing in a potassium-deficient solution than in those in a complete nutrient solution. The NO<sub>3</sub> ion increased the invertase activity, but the Cl ion inhibited it. Greatest invertase activity was found in young leaves.

BULGAKOVA, *et al.* (3) reported on diurnal fluctuations and gradients in sugars in the blades and petioles of sugar beets. They found that plants grown under a combination of electric lights and sunlight showed maximum reducing sugars in the petiole, slightly less in the veins, still less in the leaf parenchyma, and least in the roots. Sucrose increased continuously from leaf parenchyma to roots. Reducing sugars in the leaf parenchyma increased and reached their maximum towards evening. In the veins and petioles reducing sugars reached their greatest concentration in the first half of the day and decreased in the second. A close correlation between variations in the percentages of reducing sugars in the parenchyma, and the strength and duration of light suggested to these authors that reducing sugars were the first products of photosynthesis and that they were translocated to the root in that form.

BENNETT (2) observed that the curly top virus may move at rates up to 60 cm. per hour through sugar beet plants and suggests that this indicates a rapid movement of organic food materials.

#### Materials and methods

Most of the sugar beets used in this study were grown on the Northern Iowa Experimental Association farm at Kanawha, Iowa, but some were grown in the greenhouses at Ames, Iowa. In 1935 the U. S. no. 1 variety



was used and in 1936, the Pioneer variety. The plants grown on the experimental farm were spaced at 12-inch intervals in rows 22 inches apart.

Care was exercised to obtain comparable samples for chemical analyses. Border rows were not used and all collections were taken from a restricted portion of a commercial field to eliminate as much as possible the effects of soil variability. Fifteen to 20 entire beet plants were pulled and brought directly to the laboratory. One or two recently matured leaves were removed from each beet plant, the blades separated from the midribs, and 100-gm. samples of the blades weighed to  $\pm 0.10$  gm. The samples were then placed in one-quart Mason fruit jars containing 450 cc. of boiling 95 per cent. redistilled ethyl alcohol and boiled for 15 minutes. The samples were stored in the dark until analyzed, which was usually within a few weeks.

Petioles were divided into three parts: (1) The "upper" petiole consisting of the lower part of the midrib and the upper inch of the petiole proper, (2) the "middle" petiole extending from the "upper" petiole to within 3 or 4 inches of the base, and (3) the "lower" section consisting of the last 3 or 4 inches of the petiole. Only the central part of the "middle" petiole was used in all collections. The term "petiole" in the diurnal studies refers to the middle petiole section. Duplicate 75- to 100-gm. samples were weighed as before, sliced into 3- to 5-mm. pieces with a sharp knife, dropped at once into jars containing 450 cc. of boiling 95 per cent. alcohol, simmered for 20 minutes and then stored as before.

Roots were washed free from earth, and the corky layer removed. Thin transverse slices were obtained from the central part of each root. Duplicate 100-gm. samples of these slices, composited from 15 to 20 beets, were weighed as before, killed in 450 cc. of boiling 95 per cent. alcohol, allowed to simmer for 15 minutes, and then stored.

Greenhouse samples of the blades, petioles, and roots were considerably smaller than field samples. These were usually killed in 250-cc. Erlenmeyer flasks, containing 100 to 150 cc. of boiling 95 per cent. alcohol and treated as above.

#### EXPERIMENTS ON THE REVERSAL OF TRANSLOCATION

All experiments on the reversal of translocation were performed in the greenhouse. Three-months-old sugar beet plants and mother sugar beets transplanted from the field were placed in the dark room. Samples were taken at once, and at various intervals while in the dark. Samples of mature and young leaves were collected from the three-months-old plants, while only mature leaves were collected from the mother beets. Petioles were divided as in the diurnal series, except that the term "upper" petiole included both the "middle" and "upper" petiole sections. After the

three-months-old beet plants had been in the dark for a period of time, they were transferred to the light. Some of the mature leaves were bagged, using two large paper bags on each leaf, while others were left uncovered and harvested after several days. The mother sugar beet plants were left in the darkroom for a longer period of time than the three-months-old plants in order to obtain a sample of etiolated leaves. These were collected and divided in the usual manner.

The samples were of various sizes and were killed in Erlenmeyer flasks, containing boiling 95 per cent. alcohol, allowed to simmer for 10 or 15 minutes, corked, and stored for analyses.

#### METHODS OF CHEMICAL ANALYSES

The preserved material was transferred to 400-cc. beakers and usually two extractions with boiling 80 per cent. alcohol were made per day until the reducing substances had been removed from the samples as determined by tests. From 15 to 20 extractions were required. Extracts were made up to 1 liter for aliquoting.

Methods of carbohydrate analyses described by LOOMIS and SHULL (12) were used in this investigation. Reducing sugars and sucrose were determined by the MUNSON-WALKER-BERTRAND method, fructose by JACKSON and MATHEWS' modification of NYN's method, and dextrin was extracted in 10 per cent. alcohol. ✓ Sucrose was inverted by invertase.

#### Experimental data

##### MICROCHEMICAL STUDIES

Blades, petioles, and roots were studied for the presence and location of reducing sugars, sucrose, and starch. In general the tests for sucrose were not good. The Flückiger reaction was used for testing for sugars and the alcoholic IKI test for starch.

Reducing substances were found to occur in slight amounts in leaf parenchyma, but were more concentrated along the veins and in the guard cells. In the mesophyll there were sometimes a few bodies which stained with IKI.

In the petioles, longitudinal sections were more serviceable than transverse sections for determining the localization of sugars. Reducing substances were most concentrated in the parenchyma, less in the xylem and phloem, and least in the bundle cap. Sucrose was more concentrated within the veins. This relationship was also shown by macrochemical tests on dissected tissue. Starch occurred as minute grains in the starch sheath over the bundle caps and in starch-containing cells which sometimes surrounded the entire vein. These minute starch grains or bodies which stained dark with IKI were always present.

Roots contained only a small amount of reducing substances, which were most concentrated towards the periphery. Starch was not found.

#### CARBOHYDRATE CHANGES WITH PHOTOSYNTHESIS

GIRARD (9) found only slight diurnal variations of reducing sugars in the leaves of sugar beets, while variations in sucrose were marked. In the present investigation midribs and petioles were removed from the lamina, so that variations represent more closely the fluctuations within the photosynthetic mesophyll cells. Figures 1 and 2 show that diurnal variations in

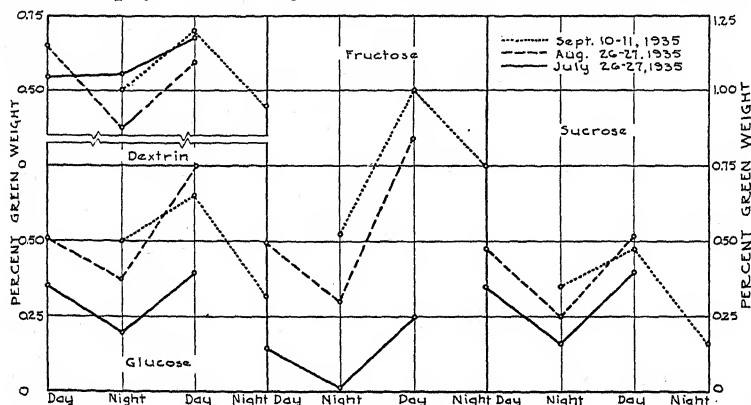


FIG. 1. Percentage and kinds of carbohydrates in leaf blades at different times during the season.

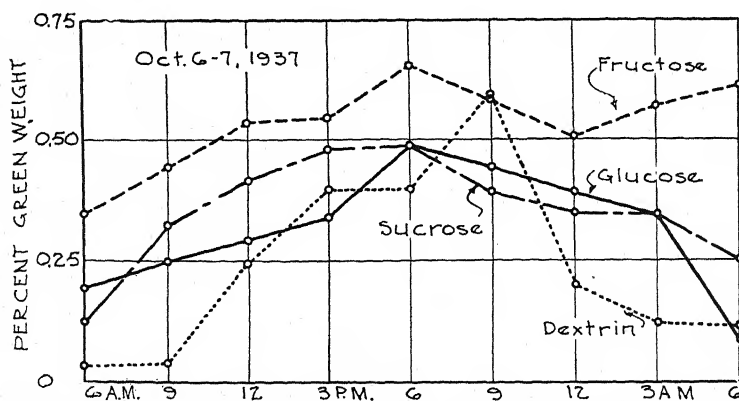


FIG. 2. Diurnal variations of carbohydrates in leaf blades.

glucose and fructose were equal to those of sucrose. Similar results were obtained in other collections, the data from which are omitted to conserve space. In figure 2 it may be observed that the sucrose curve rises more sharply in the morning than do the curves for glucose and fructose. How-

ever, in two other collections the glucose and fructose curves rose as sharply or even more so than the sucrose curves; hence one cannot tell from their early morning behavior which one, or ones, of these sugars is the first product of photosynthesis. Sucrose is not necessarily the first product of photosynthesis, as GIRARD believed (9), if the fluctuations in sucrose, as compared with the fluctuations of the hexoses, are taken as criteria.

It will be shown later that reducing sugar and sucrose levels in blades are governed by the relative activities of a sucrose-synthesizing system and invertase activity. If invertase activity is relatively low and the synthesizing system active, we may find, as in corn, mainly sucrose in the leaves; whereas, if invertase is active in the leaves, even though a sucrose-synthesizing system exists, as in the sugar beet, the sugars may be present mainly in the inverted state. This relationship is, likewise, correlated with the percentage of water in the tissue, as has been shown by the writer in work done on the sunflower (10). These relationships may explain the behavior of different plants in fluctuations of sucrose and reducing sugars during the day. It is easily conceivable that a sugar could be the first product of photosynthesis and still not fluctuate greatly in the blades. Complete absence of a sugar during periods when photosynthesis is in progress might be taken, however, as evidence that this sugar is not being formed. CLEMENTS (5), on this basis, has eliminated mannose as a product of photosynthesis.

The percentage of fructose relative to glucose increased as the season advanced (fig. 1). The response of glucose and fructose to photosynthesis did not differ greatly, however, at different seasons. It is apparent that "static" portions of sugar are present within the leaves. The *static* portions of glucose and sucrose increased only slightly as the season advanced, while that of fructose increased from none in July to a point higher than either glucose or sucrose in September. This accumulation of fructose may mean either that it was used more in growth early in the season, or that the glucose-fructose equilibrium shifted as the sugars accumulated. It may be seen from figure 1 that glucose was in excess of fructose in July. In September, fructose was in excess of glucose, and the "static" fraction was considerably greater than in July. Growth had slowed down at this time, and the beets were maturing. Young beet plants grown in the greenhouse were always found to be low in fructose. It seems that there is a distinct correlation between growth and low fructose levels within leaves. Growth was always associated, however, with a decrease in the total sugar concentration within the leaves, and this reduction may affect the glucose-fructose equilibrium. It will be shown later that fructose and glucose are interconvertible in sugar beet leaves.

Dextrin accumulated under certain conditions. Generally, young beet plants grown in the greenhouse contained a very small percentage of dex-

trin. In some field experiments dextrin was low and showed no tendency to accumulate until toward the end of the daylight hours. This condition was observed during periods when growth was rapid and sugar percentages were low. In later collections (figs. 1 and 2) dextrin showed a pronounced diurnal variation, perhaps because of the greater sugar concentration.

#### TRANSLOCATION

**NORMAL TRANSPORT.**—The three sugars, glucose, fructose, and sucrose fluctuated in the blades during the day and were often associated with similar variations in the petioles. Dextrin fluctuated in blades, but these changes were associated with no marked reactions in petioles. These results suggest that dextrin is largely hydrolyzed to glucose before it is translocated; hence the following discussion will deal more with the three sugars mentioned than with dextrin.

Figures 3 to 5 show that all of the sugars exhibited diurnal variations in the petioles. There was a large static portion of sugar within the petioles which somewhat masked variations which occurred. Table I and figure 5

TABLE I

PERCENTAGE OF THE SUGARS AND DEXTRIN IN BLADES, PETIOLES, AND ROOTS OF FIELD-GROWN SUGAR BEETS ON JULY 26-27, 1935. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PLANT PART	TIME OF COLLECTION	GLUCOSE	FRUCTOSE	SUCROSE	DEXTRIN
		%	%	%	%
Blades .....	3 P.M.	0.35	0.15	0.35	0.55
	4 A.M.	0.20	trace	0.15	0.56
	2 P.M.	0.40	0.25	0.40	0.67
Petiole .....	3 P.M.	2.30	0.48	0.45	0.35
	4 A.M.	1.88	0.38	0.30	0.37
	2 P.M.	2.50	0.48	0.45	0.35
Root .....	3 P.M.	0.16	0.00	9.20	.....
	4 A.M.	0.15	0.00	8.99	.....
	2 P.M.	0.15	0.00	9.95	.....

show, however, that there were diurnal variations which were as much as 0.5 per cent. of the green weight of the tissue.

From cross-sectional measurements of the sieve tubes it was estimated that they occupy less than 0.25 per cent. of the volume of the petiole. It is evident that even though the sieve tube contents were to fluctuate between 0 per cent. of sugar at night and saturation in the afternoon, the phloem could not have held all of the sugar observed to accumulate within the petiole. Microchemical tests always showed that reducing sugars were more concentrated in the surrounding parenchyma than in the phloem. The

writer concludes, therefore, that there was an exchange of sugars between the phloem and the surrounding cells. It is not certain that the sugars which appeared to fluctuate within the petiole were actually the sugars

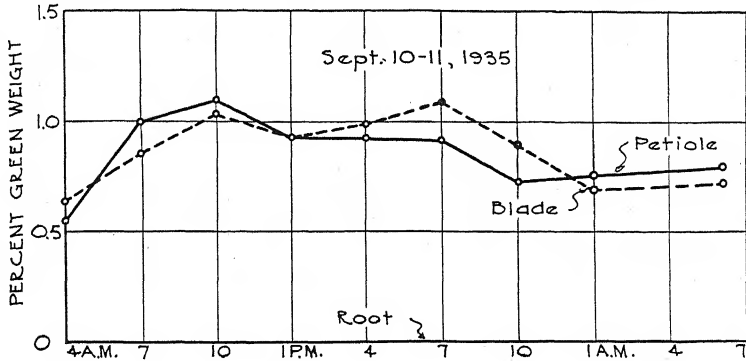


FIG. 3. Diurnal variations in fructose of blades, petioles, and roots.

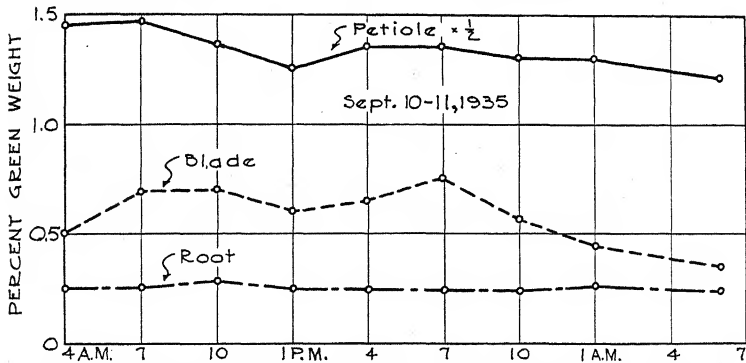


FIG. 4. Diurnal variations in glucose of blades, petioles, and roots.

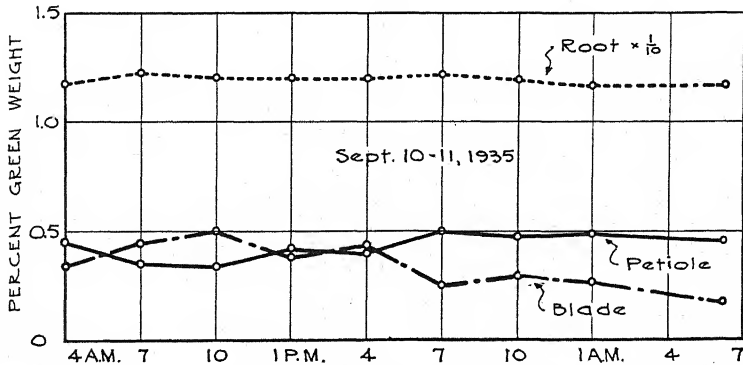


FIG. 5. Diurnal variations in sucrose of blades, petioles, and roots.

being moved. If it were not for sugar leakage from sieve tubes, there should have been no noticeable diurnal variations within the petioles. In passing from sieve tubes to the surrounding cells, sugars came into contact with various hydrolyzing, synthesizing, and transforming systems, and fluctuations of a given sugar within the entire petioles cannot be taken as evidence that this sugar was being transported in the sieve tubes of the petioles.

A close correlation between fluctuations of a sugar in the blades and in petioles probably indicates translocation, and could hardly be expected to

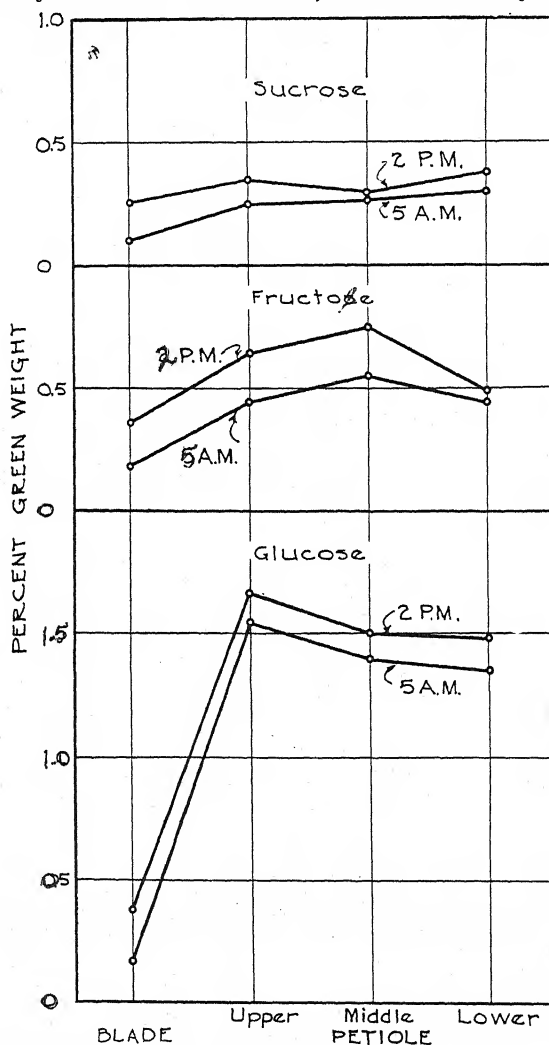


FIG. 6. Sugar gradients within sugar beet leaves in the morning and in the afternoon, September 9, 1936.

occur on the basis of chance. Such correlation in the fluctuations of fructose in blades and petioles is shown in figure 3 and it is believed to indicate fructose translocation. ENGARD (8), from ringing experiments on the red raspberry, was able to demonstrate, microchemically, that reducing sugars accumulated in the phloem above a ring and decreased to a low level below it. Cuprous oxide crystals formed in the test were localized in the phloem with only a slight precipitate in the surrounding cells. This experiment indicates that the phloem translocated reducing sugars. MASON and MASKELL (14) have shown that sucrose is a moving form in cotton. On the basis of this and other literature, and of the data presented here, it seems likely that all three sugars are translocation forms.

Figure 6 shows that all three of these sugars increased during the day and decreased by night in all parts of the petioles. Other and similar data were collected. These data strengthen the preceding in showing that sugars actually fluctuate in the petioles. There apparently is no polarization in the petiole between the phloem and the surrounding cells. During the day the concentration of the sugars increased in the phloem, this increase being followed by an increase in the surrounding cells. At night the concentration of sugars in the phloem decreased and this decrease was followed by a reversal of sugar movement.

**POLAR TRANSPORT.**—Two problems on polarity were tested. These were: (1) Do the sugars migrate out of the leaf mesophyll into the phloem in response to stimuli other than the lower sugar concentration of the latter tissue? (2) Is there, existing within the phloem of the petiole, a polarity which induces sugars to accumulate at the base of this organ? In other words, do sugars move in response to a polar condition existing within the phloem, or simply along diffusion gradients which keep the phloem filled with sugar as rapidly as it is removed into the sugar-storing cells?

Leaves, detached at the base of the petioles, were placed in beakers of water and removed to the darkroom. Table II shows a decided decrease in all three sugars in the blades. This decrease was shown to be due to respiration and movement of sugars from the blade into the petioles of the detached leaves. This experiment was repeated four times and in all cases similar results were obtained.

The transport of sugars from the blades was not sufficient to produce any marked effect within the petiole. It is significant, however, that within the petiole there was no redistribution of sugar, and no evidence of polar accumulation of sugars at the base of the petiole. The sugars must have been in a state of relatively stable equilibrium, since there was no evidence of transformation of one sugar into another, nor of synthesis or hydrolysis.

Another experiment was performed to check the above conclusions. Leaves of sugar beets were detached, placed in quart jars filled with water,



TABLE II

LOSSES OF SUGARS FROM BEET LEAVES AFTER BEING DETACHED FROM PLANTS AND PLACED IN DARK FOR 24 HOURS. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

LEAVES	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
Attached leaves (At beginning of experiment)			
Blade .....	0.32	0.57	0.36
Upper-petiole .....	2.26	0.95	0.67
Middle-petiole .....	2.25	1.00	0.60
Lower-petiole .....	2.20	0.81	0.61
Detached leaves (After 24 hours in the dark)			
Blade .....	0.11	0.38	0.12
Upper-petiole .....	2.23	0.90	0.65
Middle-petiole .....	2.23	1.00	0.57
Lower-petiole .....	2.20	0.83	0.55

and exposed to diffuse sunlight. Figure 7 shows that movement of the sugars continued, despite the fact that the leaves were detached from the plants. It is evident that the sugars moved from a region of low concentration in the blades to one of high concentration in the midribs and petioles. This experiment was repeated three times and in all cases similar results were obtained. It is of interest to note that there was no greater tendency for sugars to accumulate in one part of the petiole than in another. It seems probable that sugar was maintained at a uniform level throughout the phloem system of both leaf and petiole and that no polarity existed within the phloem system itself. The active tissue in polar transport would then become the border parenchyma of the leaf veinlets.

Dextrin showed practically no fluctuations in the petioles in the diurnal series, nor did it accumulate in the petioles of detached leaves placed in the sun as much as the sugars (fig. 7) did. Probably it was mainly hydrolyzed before it was moved.

REVERSAL EXPERIMENTS.—Young beet plants, about three months old, were placed in a darkroom. Collections of mature and young leaves were taken at once and after periods of storage. It was found that there was at first some removal from the mature leaves, but after three days the slight losses were probably caused by respiration. After five days the plants were transferred to the sunlight and some of the leaves were bagged. There was an increase in the sugars in the blades and petioles of the leaves left in the sun, but there was no increase in the bagged leaves. If there was any reversal of the translocation from the illuminated to the darkened leaves or from the roots to the darkened leaves, it was not measurable. The plants were again transferred to the darkroom. By this time the sugars had increased to the

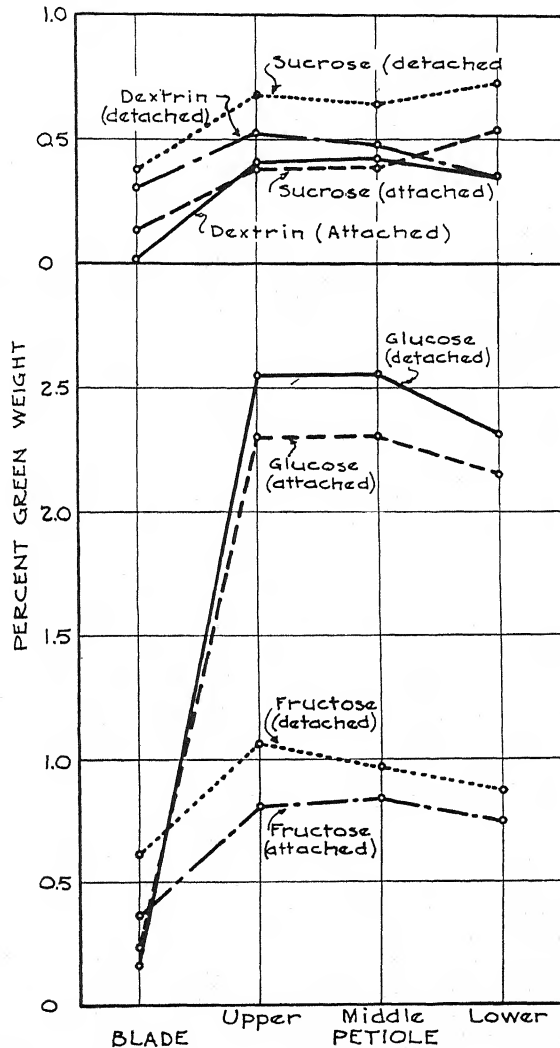


FIG. 7. Changes in the carbohydrates of detached leaves placed with their petioles in water and left in sunlight for 11 hours.

point where again there was translocation. The plants were shifted again to the light, with some of the leaves bagged (fig. 8). There was a loss of sugars from the bagged leaves, indicating no reversal of the translocatory stream. By ringing cotton plants and bagging some of the leaves, PHILLIS and MASON (17) were able to reverse the translocatory stream in leaf veins but reversal into the mesophyll was doubtful. Under their conditions the "sink" was removed. The "sink" or normal storage organ was not removed in the sugar beet experiments.

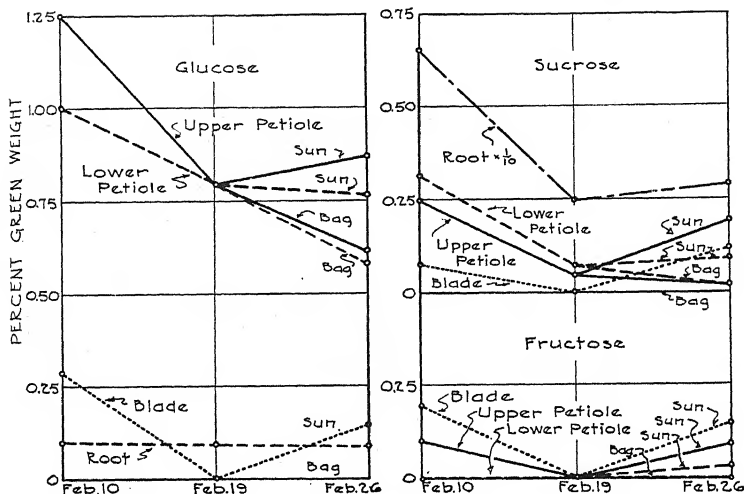


FIG. 8. Variations in the sugars of fully grown leaves after the plants were placed in the dark and then transferred to the light with some leaves bagged.

It is of interest to observe how low the concentration of the sugars in the mesophyll may drop and still have no backflow from the veins. We may see from figure 8 that the sugars were removed until they were not detected in the blades, although the concentration in the petioles was still high. Evidently the polarizing force which prevents the sugars from moving back into the mesophyll through the phloem is very strong.

Young leaves were collected and analyzed, as were the old. They were marked with India ink when placed in the dark and thus comparable leaves were collected. During the experiment the leaves more than doubled in size, yet their sugar percentage did not decrease greatly. Evidently a considerable quantity of sugar was translocated from the roots into these young leaves which is a contrast when compared with the behavior of the mature leaves, and suggests that the mechanism of polarization is not developed until late in the growth period of the leaf.

Fructose decreased to zero in all parts of the young leaf. The roots contained mainly sucrose; hence most of the sugars resulting from the reversal of translocation arose from sucrose. When sucrose is hydrolyzed by invertase, it yields equal amounts of fructose and glucose. Evidently the fructose which was formed from the hydrolysis of sucrose was either transformed into glucose or used rapidly in growth. It will be shown later that blades floated on a fructose solution converted some of the absorbed fructose into glucose. Whenever growth is rapid, fructose appears to be low, and there is evidence that this sugar may be used for synthesis to a greater extent than glucose.

Perhaps both transformation of fructose to glucose and a more rapid use of fructose in growth took place at the same time.

Sucrose was low in the young leaves and did not increase in concentration, even though it may be assumed to have moved in from the roots. Invertase is very active in young blades and petioles and probably tends to keep the sucrose in the inverted state.

GRADIENTS IN ETIOLATED LEAVES.—The etiolated leaves used in these experiments were grown in the dark from mother beet roots. These leaves differed considerably from the green leaves produced in the light. Their petioles were flattened and elongated, and the lamina were poorly developed with disproportionately large veins. The vascular bundles, however, appeared normal.

Sugar gradients and percentages found in etiolated leaves are shown in table III. As in the young leaves, fructose was low. Equal quantities of fructose and glucose should have existed after the hydrolysis of sucrose, yet the concentration of fructose was always less than that of glucose. As in the

TABLE III

PERCENTAGES OF SUGARS IN ETIOLATED LEAVES FROM MOTHER BEET PLANTS GROWN IN THE DARKROOM. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
January 29			
Blade .....	0.55	0.05	0.14
Upper-petiole .....	1.85	0.60	0.07
Middle-petiole .....	1.96	0.65	0.11
Lower-petiole .....	1.95	0.68	0.30
February 9			
Blade .....	0.80	0.27	0.34
Upper-petiole .....	1.80	0.50	0.16
Middle-petiole .....	1.70	0.80	0.18
Lower-petiole .....	2.08	0.40	0.28

TABLE IV

COMPOSITION OF YOUNG LEAVES PRODUCED ON YOUNG PLANTS IN THE DARK. CALCULATED AS PERCENTAGE OF GREEN WEIGHT

PLANT PART	GLUCOSE	FRUCTOSE	SUCROSE
	%	%	%
Blade .....	0.17	0.05	0.00
Petiole .....	1.07	0.43	0.00
Root .....	0.12	0.00	1.95

young leaves, some of the fructose was probably transformed into glucose or used in growth.

Sugar moved into the blades from the petioles. Evidently polarity differs in etiolated leaves and mature green leaves. Perhaps this difference in polarity is related to poor development of the mesophyll of etiolated leaves, or possibly to the immaturity and lack of differentiation in the etiolated leaves.

It is interesting to note that sucrose increased in concentration in passing from the petiole into the blade, thus bringing about a gradient reversal. This gradient was probably dependent, however, on the activity of the invertase and the sucrose-synthesizing systems in different parts of the leaf rather than on translocation.

Table IV shows the composition of young leaves produced in the dark on three-months-old beet plants. The sucrose content of the roots, at this stage, had been reduced to a low level for that tissue. Although sugars were moving into the young leaves from the roots, no sucrose was found in petioles or blades. Sucrose moving into the young leaves evidently was completely inverted. Perhaps this complete inversion was possible because the sugars moved into the leaves slowly as a result of low sugar level in the roots. The sugar content of the blade tissue was approximately 15 per cent. of that of the petiole and 10 per cent. of that of the root.

#### TRANSFORMATION OF SUGARS

Cut surfaces of petioles were dipped into sugar solutions. The blades were left exposed to the air to permit transpiration and draw the sugar solutions up the xylem into the leaves. Table V shows that the added sugars

TABLE V  
COMPOSITION OF BLADES AND MIDRIBS BEFORE AND AFTER BEING FED SUGAR IN  
FEEDING EXPERIMENTS

	GLUCOSE		FRUCTOSE		SUCROSE		DEXTRIN	
	PER CENT.	IN-CREASE	PER CENT.	IN-CREASE	PER CENT.	IN-CREASE	PER CENT.	IN-CREASE
Initial composition	%	%	%	%	%	%	%	%
Blades .....	0.17	.....	0.08	.....	0.20	.....	0.18	.....
Midribs .....	1.03	.....	0.00	.....	0.30	.....	0.26	.....
Fructose (3%)								
Blades .....	0.23	0.06	0.25	0.17	0.29	0.09	0.32	0.14
Midribs .....	1.63	0.60	0.39	0.39	0.69	0.39	0.38	0.12
Glucose (3%)								
Blades .....	0.30	0.13	0.22	0.14	0.35	0.15	0.34	0.16
Midribs .....	1.14	0.11	0.10	0.10	0.46	0.16	0.34	0.08
Sucrose (3%)								
Blades .....	0.55	0.38	0.40	0.32	0.31	0.11	0.49	0.31
Midribs .....	1.40	0.37	0.35	0.35	0.56	0.26	0.39	0.13

reached the leaf and that fructose was converted into glucose and glucose into fructose. The conversion of fructose into glucose was the more complete reaction, however. The dextrin-synthesizing mechanism was active. The sucrose response was not great, but here too there was synthesis.

Sucrose was hydrolyzed to the equilibrium point existing between the tissues as rapidly as it was absorbed and sucrose concentration was about the same whether the leaves were fed with the invert sugars or with sucrose.

Invertase was found to be very active in sugar beet blades and probably was responsible for the strong hydrolysis of the sucrose. Leaves fed either glucose or fructose synthesized some sucrose, but the synthesis was not strong. NURMIA (15) showed that leaves free or almost free of invertase were able to synthesize sucrose strongly. Evidently invertase is associated with sucrose inversion and not with sucrose synthesis. More evidence relating to this subject will be reported in another paper.

### Discussion

Sugars migrated in detached mature leaves from the blades into the petioles, whether the leaves were placed in the sun or in the dark. This result is interpreted as indicating that sugars move from the blade mesophyll into the phloem in a polar direction. No suggestion is offered as to the mechanism involved. It is obvious that an expenditure of energy is necessary to move sugars from a region of low sugar content in the blades to a region of high concentration in the petioles. The polarizing force is strong, since blades were often found to contain no detectable sugar, even though sugars were present in the petiole in considerable percentages.

As long as there was a "sink" in the root of the sugar beets, the sugars traveled in that direction from the fully grown leaves. The use of carbohydrates seems to be of importance in determining the direction of translocation. Young leaves imported sugars readily, probably because the machinery of polarized movement had not become established. The leaves from young or rapidly growing sugar beet plants were always low in fructose, while the leaves from older or mature plants were always high in fructose. This condition could have been brought about entirely by factors affecting the glucose-fructose equilibrium. On the other hand, it is possible that fructose was used preferentially in growth. Since cellulose and other structural materials are considered to be glucosans, fructose may have been more readily transformed into "active" glucose than was glucose itself.

It is apparent that the relative proportions of the various carbohydrates in plant tissues are governed by a variety of transforming systems, as well as by other factors. Some of the factors which might be involved in determining the relative amounts of different carbohydrates within tissues are as follows: (1) The activities of the various carbohydrate-transforming sys-

tems; (2) the distribution of the carbohydrates within the cell; (3) the distribution of the carbohydrate-transforming systems within the cell; (4) the time available for the reactions; (5) the temperature; (6) growth and respiration; and (7) translocation.

The feeding experiments indicated that the various sugars were readily transformed into other forms. Leaves fed sucrose contained no more sucrose than those fed either glucose or fructose. If sucrose were formed by photosynthesis in the leaf instead of introduced artificially, we would expect the same degree of hydrolysis. In the same manner, if one of the reducing sugars was the first sugar of photosynthesis, we would expect it to be polymerized to the normal equilibrium point. Sugar transformations within the tissue prevent the use of diurnal accumulations as evidence that particular forms are produced in photosynthesis or are moved in a given transport system.

### Summary

1. The sugar beet was used in studies on photosynthesis, translocation, and transformation of the sugars. It was found that fructose increased in the blades as the season progressed. A low level of fructose was associated with rapid growth and a higher level with slow growth and maturity of the plant.

2. Fructose, glucose, and sucrose all showed marked diurnal variations within the blades. Variations in fructose and glucose together were usually twice those of sucrose. Dextrin at times showed marked diurnal variations within the blades and is an important secondary product of photosynthesis.

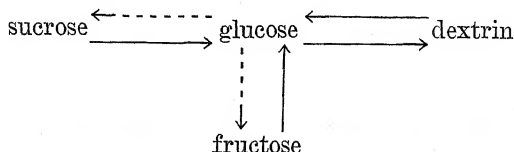
3. The sugars in mature leaves placed in the dark, whether the leaves were detached or on growing plants, continued to move out of the blades into the petioles until the blades were depleted of sugars. Evidently sugars move out of the blade mesophyll into the veins in a polar direction. There was, however, no tendency for any of the sugars from detached leaves to accumulate at the base of the petiole. Polarization thus appears to be a property of the border parenchyma of the blades.

4. Sugars of detached leaves placed in the sun moved out of the blade mesophyll and accumulated more or less evenly throughout the midribs and petioles. Again there was no tendency for any of the sugars to accumulate more in one section of the petiole than in another.

5. While there was a polarity which prevented the sugars from the phloem moving back into the blade mesophyll, no such polarity existed between the phloem and parenchyma of the petioles. During the daylight hours sugar passed out of the phloem and accumulated in the surrounding cells, while at night the reverse movement took place.

6. Etiolated leaves of beet plants contained considerably more glucose than fructose. Practically all of the sugar present in these leaves arose from sucrose.

7. In feeding experiments, fructose was converted into glucose and glucose into dextrin. There was some conversion of glucose into fructose. The diagram below, in a general way, illustrates the observed reactions. The broken lines indicate the weaker reactions.



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# FOLIAR DIAGNOSIS: THE INFLUENCE OF THE SOIL ON THE ACTION OF FERTILIZERS<sup>1</sup>

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(WITH TWELVE FIGURES)

## Introduction

In an earlier paper (6) reference was made to the fact that no piece of land, however carefully selected for experimental purposes, is absolutely uniform. If the area is divided into equal sections and the sections sub-divided into plots of the same size, the yields from similarly treated duplicate plots planted on the same day with selected homogeneous seed will rarely be the same, and not infrequently will be widely divergent. For example, the relative percentage differences in yields—i.e. the value of  $\left(\frac{x-y}{x} \times 100\right)$  where x and y are the respective yields of one pair of similarly treated plots—may differ by less than 5 per cent., whereas that of another pair may differ by 50 per cent. or more.

The relationship of the foliar diagnosis to the yields of potato plants growing on differently fertilized plots of tiers 1 and 2 of the vegetable fertility plots of the Pennsylvania Agricultural Experiment Station have been described in three earlier papers (6, 7, 8), familiarity with which is assumed in the present report. It was shown (6, 8) that the foliar diagnosis of a particular plot treatment was a characteristic of that plot and is related to the nature of the fertilizer applied and also to the development and yield of the plants growing thereon.

The object of the present investigation is to apply the method of foliar diagnosis (6) to an examination of the nature of the influence of the soil on the action of fertilizers, with special reference to plants of the same species growing on (1) a homogeneous soil and (2) a heterogeneous soil.

It has been repeatedly shown, (1, 2, 8), that when soils of two similarly treated plots are relatively homogeneous, as deduced from similar development and yields of the plants growing thereon, duplicate pairs will, in general, and with a sufficient degree of approximation for field experiments, be represented by similar foliar diagnosis. On the other hand, when the soils of two similarly treated plots are not uniform, as indicated by widely different development and yields of the plants growing thereon, they will be represented by dissimilar foliar diagnosis.

Consequently, markedly dissimilar foliar diagnoses of plants of the same species from plots treated alike is an indication of the heterogeneity of the

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soils of the plots under comparison. These soil effects from duplicate plots may be the result not only of chemical differences but also of physical differences which may produce differences in the water or air supply to the roots.

### Materials and methods

The present study was carried out on potato plants growing on plots from tiers, 1, 2, 4, and 5 of section B of the long-continued vegetable fertility experiments of the Pennsylvania Agricultural Experiment Station (4). The description of the experimental layout has been given by one of us (4). The analytical methods adopted were those described in an earlier paper (6).

### Presentation of results

The treatment and yields of tubers from the duplicate plots are shown in table I. Tier 4 is the duplicate of tier 1, and tier 5 the duplicate of tier 2. The fourth column shows the relative differences in the yields of the respective duplicates in tiers 4 and 5 expressed as a percentage of the yield of the duplicate in tiers 1 and 2, respectively. The fifth and sixth columns show the mean *intensity of nutrition* and the mean *NPK-unit* of the fourth leaf (6). In the first column (A) designates the plots in tiers 1 and 2, and (B) those in tiers 4 and 5. The nitrogen, phosphoric acid, and potash contents of the fourth leaf from the base of the stalk at the various sampling dates, expressed (1) as percentage of each element in the dried foliage, (2) in milligram equivalents, and (3) as the composition of the *NPK-unit* are presented in tables II and III and are shown graphically in figures 1 to 12. Figure 1 indicates the percentages of N,  $P_2O_5$ , and  $K_2O$  in the dried foliage as ordinates, and dates of sampling as abscissae. In figures 2 to 10 the *NPK-units*—i.e. the  $(N-P_2O_5-K_2O)$  equilibria at the respective sampling dates—are plotted in trilinear coordinates, and the mean *NPK-unit* in figures 11 and 12.

### Discussion and interpretation of results

The changes in the signs (+, -) in table I indicate that the differences in the yields of duplicate plots are not caused by a factor operating uniformly over the whole area. Thus, there are seven plots in tiers 1 and 2 in which the yield is greater (+) than that of the duplicate in tier 4 or 5, and six plots in which the yields in tiers 1 and 2 are less than (-) the respective duplicates in tier 4 or 5.

There are six plots in which the differences in the yields of duplicate plots are less than 10 per cent. and which may be regarded as falling within the degree of approximation that would occur in a relatively uniform soil. The relative percentage differences in yields  $\left(\frac{x-y}{x} \times 100\right)$  of the remaining duplicate plots range from -12.9 to +54.8 per cent., showing that greater

TABLE I

RELATIVE PERCENTAGE DIFFERENCES IN YIELDS BETWEEN DUPLICATE PLOTS TOGETHER  
WITH THE MEAN INTENSITY OF NUTRITION AND THE MEAN NPK-UNIT

TREATMENT SYMBOL	TIER	PLOT	YIELDS	RELATIVE PERCENTAGE DIFFERENCE IN YIELDS	MEAN INTEN- SITY OF NUTRITION (TOTAL N + P <sub>2</sub> O <sub>5</sub> + K <sub>2</sub> O)	MEAN NPK-UNIT
2(NPK)	(A)	2	16	lb.		
	(B)	5	2	170	%	
0.5(NPK)	(A)	2	14	150	+ 4.7	11.45
	(B)	5	4	158	- 5.3	10.66
(1.5N)PK	(A)	2	4	157		8.79
	(B)	5	14	148	+ 5.7	8.93
NK	(A)	1	7	163		9.74
	(B)	4	11	174	- 6.7	10.39
Manure	(A)	1	15	196		10.22
	(B)	4	3	182	+ 7.2	10.91
NP(1.5K)	(A)	2	12	174		10.33
	(B)	5	6	157	+ 9.8	10.29
NP	(A)	1	6	124		10.74
	(B)	4	12	140	- 12.9	10.74
N	(A)	1	2	109		6.10
	(B)	4	16	88	+ 19.2	7.40
N(1.5P)K	(A)	2	8	176		9.62
	(B)	5	10	210	- 19.4	9.70
NPK	(A)	1	10	162		9.39
	(B)	4	8	195	- 20.3	10.03
P	(A)	1	3	114		5.65
	(B)	4	15	88	+ 22.8	7.28
PK	(A)	1	8	148		9.58
	(B)	4	10	208	- 40.5	9.50
K	(A)	1	4	155		9.48
	(B)	4	14	70	+ 54.8	9.32

TABLE II

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P<sub>2</sub>O<sub>5</sub> AND K<sub>2</sub>O IN DRY FOLIAGE AND THE SUM N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS  
TTERS 1 AND 2

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE					MILLIGRAM EQUIVALENTS					COMPOSITION OF THE NPK-UNIT				
			N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	M <sub>x</sub> + M <sub>y</sub> + M <sub>z</sub>	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>	S	X	Y	Z			
			M <sub>x</sub>	M <sub>y</sub>	M <sub>z</sub>	s									E <sub>x</sub>	E <sub>y</sub>	E <sub>z</sub>
July 7	2(NPK)	2 No. 16	5.23	0.682	8.771	14.683	mg. eq.	373.422	28.849	186.822	mg. eq.	589.093	%	63.389	4.897	%	31.713
July 29			4.04	0.562	5.930	10.532	288.456	23.773	126.309	438.538	65.777	5.420	28.802				
August 9			3.87	0.500	6.124	10.494	276.318	21.150	130.441	427.909	64.574	4.943	30.483				
August 24	0.5(NPK)	2 No. 14	3.50	0.354	6.453	10.107	235.620	14.974	137.449	388.043	60.720	3.858	35.422				
July 7			4.84	0.520	6.108	11.468	345.576	21.996	130.100	497.672	69.439	4.418	26.142				
July 29			3.77	0.528	4.379	8.677	269.178	22.334	93.273	384.785	69.957	5.803	24.239				
August 9	(1.5 N)PK	2 No. 4	3.84	0.466	4.031	7.837	238.476	19.712	85.860	344.048	69.315	5.729	24.956				
August 24			2.84	0.418	3.920	7.178	202.776	17.681	83.496	303.953	66.715	5.817	27.468				
July 7			5.23	0.614	6.780	12.624	373.422	25.972	144.414	543.808	68.667	4.775	24.555				
July 29	NK	1 No. 7	4.00	0.488	5.040	9.528	285.600	20.642	107.352	413.594	69.054	4.990	24.956				
August 9			3.98	0.464	4.534	8.978	284.172	19.627	96.574	400.373	70.977	4.903	24.120				
August 24			3.16	0.356	4.302	7.818	225.624	15.059	91.633	332.316	67.892	4.532	27.572				
July 7	Manure	1 No. 15	5.10	0.464	6.630	12.194	364.140	19.627	141.176	524.943	69.367	3.739	26.894				
July 29			4.03	0.510	5.550	10.090	287.742	21.573	118.300	427.615	67.290	5.045	27.665				
August 9			3.78	0.452	5.280	9.512	269.892	19.119	112.443	401.454	67.229	4.763	28.008				
August 24	NP(1.5K)	2 No. 12	3.26	0.352	5.460	9.072	232.764	14.889	116.234	363.887	63.964	4.092	31.941				
July 7			4.65	0.688	7.550	12.888	332.010	29.102	160.815	521.927	63.612	5.575	30.812				
July 29			3.80	0.608	5.128	9.536	271.320	25.718	109.226	406.264	66.785	6.331	26.887				
August 9	NP	1 No. 6	3.79	0.564	5.155	9.509	270.606	23.857	109.801	404.264	66.939	5.902	27.161				
August 24			3.28	0.448	5.658	9.386	234.192	18.950	120.515	373.657	62.675	5.071	32.254				
July 7			4.86	0.594	7.798	13.252	347.004	25.126	166.097	538.227	64.470	4.669	30.860				
July 29	NP	2 No. 6	4.19	0.522	5.767	10.479	299.166	22.081	122.837	444.084	67.368	4.972	24.662				
August 9			3.71	0.513	5.782	10.005	264.894	21.699	123.157	409.750	64.647	5.296	30.057				
August 24			2.82	0.394	5.996	9.210	201.348	16.666	127.715	345.729	58.239	4.822	36.939				
July 7	NP	1 No. 6	5.14	0.590	2.530	8.260	366.996	24.957	53.910	445.863	82.311	5.598	12.091				
July 29			3.87	0.532	1.520	5.922	276.318	22.504	32.355	331.177	83.435	6.794	9.771				
August 9			3.71	0.486	1.190	5.386	264.894	20.558	25.262	310.714	85.253	6.617	8.130				
August 24			3.36	0.390	1.090	4.840	239.904	16.751	23.110	279.765	85.752	5.987	8.261				

TABLE II—(Continued)

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P<sub>2</sub>O<sub>5</sub> AND K<sub>2</sub>O IN DRY FOLIAGE AND THE SUM N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS  
TIERS 1 AND 2

LITERS 1 AND 2																
DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE					MILLIGRAM EQUIVALENTS					COMPOSITION OF THE NPK-UNIT			
			N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	M <sub>x</sub> + M <sub>y</sub> + M <sub>z</sub>	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>	X	Y	Z			
			M <sub>x</sub>	M <sub>y</sub>	M <sub>z</sub>	s	E <sub>x</sub>	E <sub>y</sub>	E <sub>z</sub>	S	( $\frac{E_x}{100 S}$ )	( $\frac{E_y}{100 S}$ )	( $\frac{E_z}{100 S}$ )			
July 7	N	1 No. 2	%	%	%	%	mg. eq.	mg. eq.	mg. eq.	mg. eq.	%	%	%			
July 29			5.12	0.404	3.970	9.494	365.568	17.089	84.561	467.218	78.244	3.658	18.098			
August 9			4.42	0.534	2.540	7.494	315.588	22.588	54.102	392.278	80.450	5.759	13.791			
August 24			4.15	0.472	2.010	6.632	296.310	19.966	42.813	359.089	82.517	5.561	11.922			
July 7	N(1.5P)K	2 No. 8	3.64	0.344	1.770	5.754	259.896	14.551	37.701	312.148	83.261	4.661	12.078			
July 29			4.84	0.710	6.969	12.519	345.576	30.033	148.440	524.049	65.944	5.730	28.325			
August 9			3.72	0.564	4.914	9.198	256.608	23.857	104.668	394.133	67.391	6.054	26.557			
August 24			3.46	0.482	4.934	8.876	247.004	20.389	105.094	372.527	66.314	5.473	28.209			
July 7	NPK	1 No. 10	2.86	0.350	4.670	7.880	204.204	14.805	99.471	318.480	64.117	4.650	31.233			
July 29			4.98	0.584	6.589	12.153	355.570	24.70	140.34	520.62	68.30	4.74	26.96			
August 9			3.88	0.509	4.337	8.726	277.03	21.53	92.38	390.94	70.86	5.51	23.63			
August 24			3.62	0.482	4.697	8.799	258.47	20.39	100.05	376.90	68.22	5.38	26.40			
July 7	P	1 No. 3	3.14	0.392	4.348	7.880	224.19	16.58	92.61	333.39	67.25	4.97	27.78			
July 29			4.57	0.550	2.41	7.530	326.298	25.042	51.333	402.673	81.034	6.218	12.747			
August 9			3.46	0.521	1.64	5.621	247.044	22.038	34.932	304.014	81.260	7.250	11.490			
August 24			3.24	0.520	1.29	5.050	231.336	21.996	27.477	280.809	82.383	7.831	9.786			
July 7	PK	1 No. 8	2.79	0.450	1.11	4.350	199.206	19.035	26.643	241.884	82.359	7.868	9.773			
July 29			4.55	0.620	6.76	11.930	324.870	26.395	143.967	495.232	65.599	5.330	29.071			
August 9			3.36	0.510	5.32	9.190	239.904	21.573	113.337	374.814	64.006	5.755	30.239			
August 24			3.08	0.490	5.35	8.920	219.912	20.980	113.912	354.804	61.981	5.913	32.105			
July 7	K	1 No. 4	2.82	0.430	5.04	8.290	201.348	18.189	107.309	326.846	61.603	5.565	32.832			
July 29			4.61	0.424	6.32	11.354	329.154	17.935	134.573	481.662	68.336	3.725	27.939			
August 9			3.87	0.540	5.22	9.630	276.318	22.842	111.122	410.282	67.349	5.567	27.084			
August 24			3.39	0.464	4.91	8.764	242.046	19.627	104.689	366.362	66.068	5.358	28.575			
August 24			2.78	0.370	5.00	8.150	198.492	15.651	106.500	320.643	61.904	4.881	33.215			

TABLE III

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P<sub>2</sub>O<sub>5</sub> AND K<sub>2</sub>O IN DRY FOLIAGE AND THE SUM N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS  
DUPLICATE PLOTS TIERS 4 AND 5

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE					MILLIGRAM EQUIVALENTS					COMPOSITION OF THE NPK-UNIT			
			N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	M <sub>x</sub> + M <sub>y</sub> + M <sub>z</sub>	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>	X	Y	Z			
			M <sub>x</sub>	M <sub>y</sub>	M <sub>z</sub>	s										
			%	%	%	%	E <sub>x</sub>	E <sub>y</sub>	E <sub>z</sub>	S	100 $\frac{E_x}{S}$	100 $\frac{E_y}{S}$	100 $\frac{E_z}{S}$	%	%	%
July 7	2 (NPK)	5 No. 2	5.03	0.650	7.521	13.201	359.142	27.495	160.197	mg. eq. 546.834	65.676	5.028	29.292			
July 29			4.08	0.509	5.542	10.131	291.312	21.531	118.045	430.888	67.607	4.997	27.396			
August 9			3.76	0.454	5.658	9.872	268.464	19.204	120.515	408.183	65.770	4.705	29.525			
August 24			3.35	0.368	5.775	9.493	239.190	15.566	123.007	377.763	63.317	4.120	32.562			
July 7	0.5 (NPK)	5 No. 4	4.34	0.524	6.705	11.569	309.876	22.165	142.816	474.857	65.257	4.668	30.075			
July 29			3.57	0.486	4.883	8.939	254.898	20.558	104.008	379.464	67.173	5.418	27.409			
August 9			3.12	0.454	4.310	7.884	222.768	19.204	91.803	333.775	66.742	5.753	27.504			
August 24			2.75	0.400	4.162	7.312	196.350	16.920	88.651	301.921	65.033	5.604	29.362			
July 7	(1.5N) PK	5 No. 14	4.98	0.528	6.814	12.322	355.572	22.334	145.138	523.044	67.981	4.270	27.748			
July 29			4.26	0.446	5.744	10.450	304.164	18.866	122.347	445.377	68.293	4.235	29.470			
August 9			3.76	0.430	5.705	9.895	268.464	18.189	121.516	408.169	65.772	4.456	29.771			
August 24			3.23	0.348	5.310	8.888	230.622	14.720	113.103	358.445	64.339	4.106	31.553			
July 7	NK	4 No. 11	5.04	0.412	6.790	12.242	359.856	17.428	144.627	521.911	68.949	3.339	27.711			
July 29			4.31	0.498	5.968	10.776	307.734	21.065	127.118	455.917	67.498	4.620	27.882			
August 9			3.78	0.420	6.269	10.469	269.892	17.766	133.550	421.188	64.079	4.218	31.703			
August 24			3.36	0.332	6.434	10.126	239.904	14.044	137.044	390.992	61.358	3.592	35.050			
July 7	Manure	4 No. 3	4.84	0.750	7.248	12.838	345.576	31.725	154.382	531.683	64.997	5.967	29.036			
July 29			4.04	0.604	5.116	9.760	288.456	25.549	108.971	422.976	68.197	6.040	25.763			
August 9			3.58	0.502	4.988	9.070	255.612	21.235	106.244	383.091	66.723	5.543	27.733			
August 24			3.45	0.472	5.601	9.523	246.330	19.966	119.301	385.597	63.883	5.178	30.939			
July 7	NP (1.5K)	5 No. 6	4.54	0.554	6.794	11.888	324.156	23.434	144.712	492.302	65.845	4.760	29.395			
July 29			3.71	0.502	5.124	9.336	264.894	21.235	109.141	395.270	67.015	5.372	27.612			
August 9			3.26	0.444	4.653	8.357	232.764	18.781	99.109	350.654	66.379	5.356	28.272			
August 24			3.01	0.382	5.310	8.702	214.914	16.159	113.103	344.176	62.443	4.695	32.862			
July 7	NP	4 No. 12	5.08	0.558	4.632	10.270	362.712	23.603	98.662	484.977	74.789	4.867	20.344			
July 29			4.20	0.526	2.635	7.361	299.880	22.249	56.125	378.254	79.280	5.882	14.838			
August 9			3.78	0.480	2.170	6.430	269.892	20.304	46.231	336.417	80.225	6.035	13.739			
August 24			3.37	0.412	1.755	5.537	240.618	17.428	37.381	295.427	81.447	5.899	12.653			

TABLE III—(Continued)

COMPOSITION OF THE FOURTH LEAF AT PERIODIC INTERVALS IN TERMS OF PERCENTAGES OF N, P<sub>2</sub>O<sub>5</sub> AND K<sub>2</sub>O IN DRY FOLIAGE AND THE SUM N + P<sub>2</sub>O<sub>5</sub> + K<sub>2</sub>O TOGETHER WITH THE MILLIGRAM EQUIVALENTS, AND THE NPK-UNITS  
DUPLICATE PLOTS TIERS 4 AND 5

DATE OF SAMPLING	TREATMENT	TIER AND PLOT	DRIED FOLIAGE					MILLIGRAM EQUIVALENTS					COMPOSITION OF THE NPK-UNIT			
			N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	M <sub>x</sub> + M <sub>y</sub> + M <sub>z</sub>	N	P <sub>2</sub> O <sub>5</sub>	K <sub>2</sub> O	E <sub>x</sub> + E <sub>y</sub> + E <sub>z</sub>	X	Y	Z			
			M <sub>x</sub>	M <sub>y</sub>	M <sub>z</sub>	s	E <sub>x</sub>	E <sub>y</sub>	E <sub>z</sub>	S	$\frac{E_x}{100 \cdot S}$	$\frac{E_y}{100 \cdot S}$	$\frac{E_z}{100 \cdot S}$			
			%	%	%	%	mg. eq.	mg. eq.	mg. eq.	mg. eq.	%	%	%			
July 7	N	4 No. 16	4.71	0.424	4.438	9.572	336.294	17.935	94.529	448.758	74.939	3.996	21.064			
July 29			4.11	0.502	3.139	7.751	293.454	21.235	66.861	381.550	76.911	5.565	17.523			
August 9			3.75	0.458	2.635	6.843	267.750	19.373	56.125	343.348	78.005	5.644	16.351			
August 24			3.40	0.362	2.151	5.913	242.760	15.313	45.816	303.889	79.884	5.039	15.076			
July 7	N(15P)K	5 No. 10	4.84	0.658	6.678	12.176	345.576	27.833	142.241	515.650	67.017	5.397	27.584			
July 29			3.90	0.608	5.031	9.539	278.460	25.718	107.160	411.338	67.696	6.252	26.051			
August 9			3.40	0.476	4.883	8.759	242.760	20.135	104.008	366.903	66.164	5.488	28.348			
August 24			2.96	0.404	4.961	8.325	211.344	17.089	105.669	334.102	63.257	5.115	31.628			
July 7	NPK	4 No. 8	4.92	0.604	6.907	12.431	351.388	25.549	147.119	523.956	67.043	4.876	28.078			
July 29			4.36	0.542	5.129	10.081	311.304	22.927	109.248	443.479	70.196	5.169	24.634			
August 9			3.62	0.460	5.000	9.080	258.468	19.458	106.500	384.426	67.209	5.061	27.704			
August 24			3.23	0.398	4.961	8.589	230.822	16.835	105.669	353.126	65.309	4.767	29.924			
July 7	P	4 No. 15	4.30	0.548	5.155	10.003	307.020	23.180	109.695	439.895	69.794	5.269	24.937			
July 29			3.38	0.512	3.488	7.380	241.332	21.658	74.294	337.284	71.551	6.421	22.027			
August 9			3.06	0.514	3.042	6.616	218.484	21.742	64.752	304.978	71.639	7.129	21.232			
August 24			2.34	0.444	2.352	5.136	167.076	18.781	50.055	235.912	70.821	7.961	21.218			
July 7	PK	4 No. 10	4.45	0.636	6.651	11.737	317.730	26.903	141.666	486.299	65.336	5.532	29.131			
July 29			3.64	0.580	5.193	9.413	259.896	24.534	110.611	395.041	65.789	6.210	27.999			
August 9			3.10	0.484	4.872	8.456	221.340	20.473	103.773	345.586	64.048	5.924	30.028			
August 24			2.75	0.370	5.300	8.420	196.350	15.651	112.890	324.891	60.436	4.817	34.131			
July 7	K	4 No. 14	4.10	0.432	6.317	10.849	292.740	18.274	134.552	445.566	65.701	4.101	30.198			
July 29			3.50	0.452	5.232	9.184	249.900	19.119	111.442	380.461	65.693	5.025	29.291			
August 9			3.20	0.432	5.496	9.128	228.480	18.274	117.065	363.819	62.800	5.023	32.177			
August 24			2.56	0.328	5.242	8.130	182.784	13.874	111.655	308.313	59.285	4.499	36.215			



differences exist in the soils of these like-treated pairs of plots than in the other groups of six plots.

The highest difference between the yields of duplicate plots is that between the K plots (no. 1-4 and no. 4-14). This is associated with a peculiar phenomenon which accompanies the absorption of potassium (*i.e.*, potash nutrition), which will be discussed in later papers.

GRAPHIC REPRESENTATION OF PERCENTAGES OF N,  $P_2O_5$  AND  $K_2O$  IN  
THE DRIED FOLIAGE AS ORDINATES AND DATES OF SAMPLING  
AS ABSCISSAE

The method of interpreting such graphs has been given in earlier papers (6, 7, 8). An inspection of the graphs (fig. 1) shows that much greater dif-

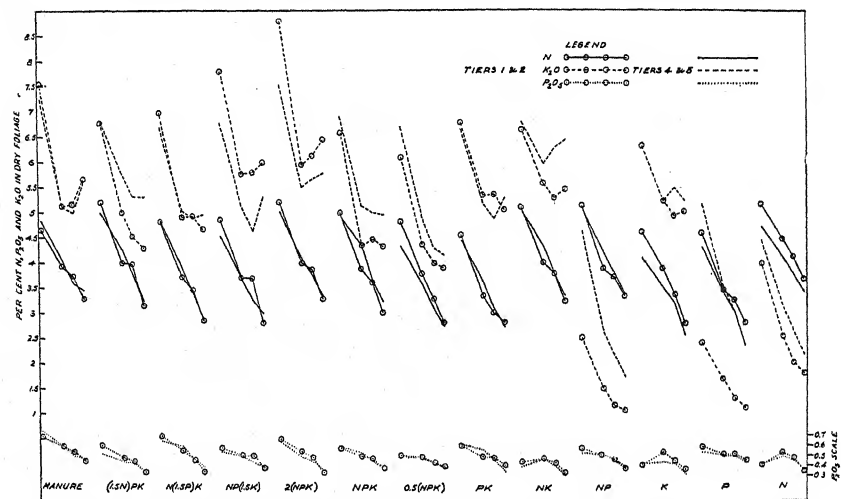


FIG. 1. The percentages of N,  $P_2O_5$  and  $K_2O$  in the fourth leaf with advancing age.

ferences occur in the potash content of couplets, with advancing age of the leaf, than in the nitrogen or phosphoric acid contents. In two couplets not receiving any mineral potash applications (P and NP) the differences are phenomenal, showing the marked differences in the character of the soil of these couplets and its influence on the K nutrition of the plant.

The characteristics of the graphs in figure 1 which appear most frequently in connection with differences in yield of duplicate plots are the following:

Among the couplets with relatively high yields (average 150 pounds or more) which differed less than 10 per cent., the plants growing on the plot with greater yield are characterized by a more rapid utilization of  $K_2O$  at a higher level than those from the lower yielding plot, especially during the early part of the sampling period. Exceptions are (1.5N)PK, in which,

though utilization is greater throughout the season in the duplicate with highest yield, the level of  $K_2O$  is lower at the end of the sampling period; and NK, in which the plot with the lowest yield, (no. 1-7), has higher utilization at the beginning of the sampling period, but reaches a lower level at the second sampling date.

Among couplets with relatively low yields (average less than 150 pounds) or with high yields differing by more than 10 per cent., more rapid utilization of  $K_2O$  during part or all of the season at a higher level is more often associated with lower yield. An exception is NP, in which the more rapid utilization at the higher level characterizes the plot (no. 4-12) with the greater yield.

The form of the  $K_2O$  graphs is similar for duplicate plots of which the yields differ less than 10 per cent., with the exception of NK, in the respect that utilization is very rapid at the beginning, and is distinctly slower at the end of the sampling period; accumulation sometimes occurs at the latter time.

The form of  $K_2O$  graphs is dissimilar for duplicate plots of which the yields differ more than 10 per cent. from each other, except in NP, P, and N, in the first two of which the contents of  $K_2O$  in the leaf are markedly different in the duplicate plots.

No consistent relation between either N,  $P_2O_5$ , or the balance between N and  $P_2O_5$  utilization (5) and the yield of duplicates may be observed.

#### GRAPHIC REPRESENTATION OF THE NPK-UNITS IN TRILINEAR COORDINATES

COURSE OF NUTRITION WITH RESPECT TO NITROGEN, PHOSPHORIC ACID, AND POTASH FROM ONE SAMPLING DATE TO ANOTHER.—The method of deriving the *NPK-unit* and of interpreting the graphs has been given in an earlier paper (6). Displacement upwards toward the summit of the triangle ( $N=100$  per cent.) indicates an increase in the nitrogen of the *NPK-unit*; displacement to the left base apex ( $K_2O=100$  per cent.), an increase in the potash content of the *NPK-unit*; and displacement towards the right base apex ( $P_2O_5=100$  per cent.), an increase in the  $P_2O_5$  content of the *NPK-unit*. The solid lines (figs. 2-10) indicate the equilibrium between nitrogen, phosphoric acid, and potash at successive dates of sampling (indicated by the numerals 1, 2, 3, 4) of the fourth leaf of plants growing on tiers 1 and 2 and marked (A) in table I, and the broken lines indicate that of the respective duplicate plots in tiers 4 and 5, marked (B) in table I.

It should be kept in mind that a fertilizer may intervene to produce (a) a change in the *intensity of nutrition* or (b) a change in the  $N-P_2O_5-K_2O$  equilibrium (*NPK-unit*) or a change in (a) and (b) simultaneously (6).

The eye will be able to follow the differences in the  $N-P_2O_5-K_2O$  equilibrium between the duplicate pairs, with the advancing age of the leaf, without the necessity of any detailed description of these differences.

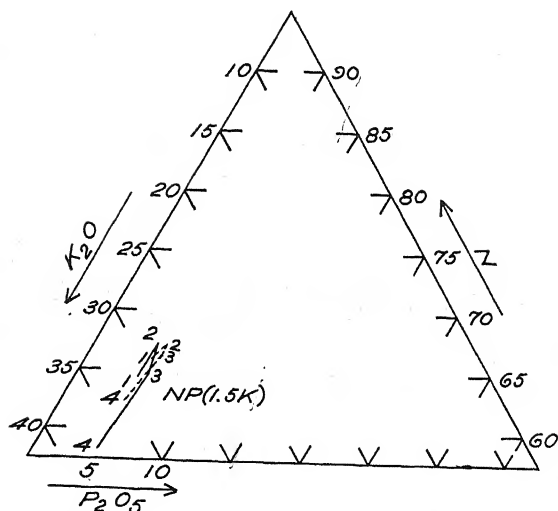


FIG. 2. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [NP(1.5K)].

The fact that the *NPK-unit* in no case ever follows the direction first taken is indicated by the zig-zag appearance of the graphs. The changes in the N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O equilibrium of a particular plot graph from one sampling date to another are the result of meteorological influences during growth and development, and the different forms of the graphs show that this factor does not act identically over all treatments.

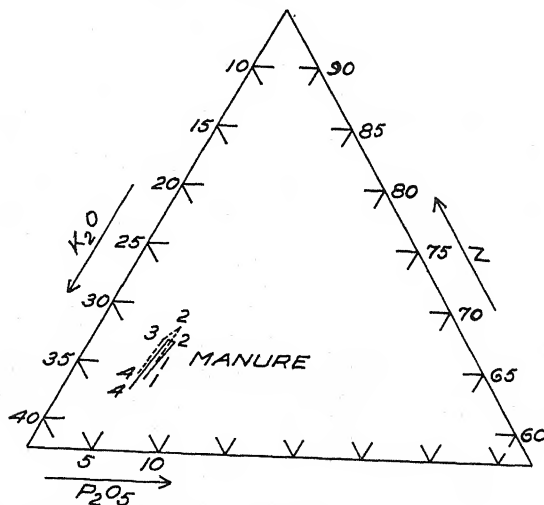


FIG. 3. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates (manure).

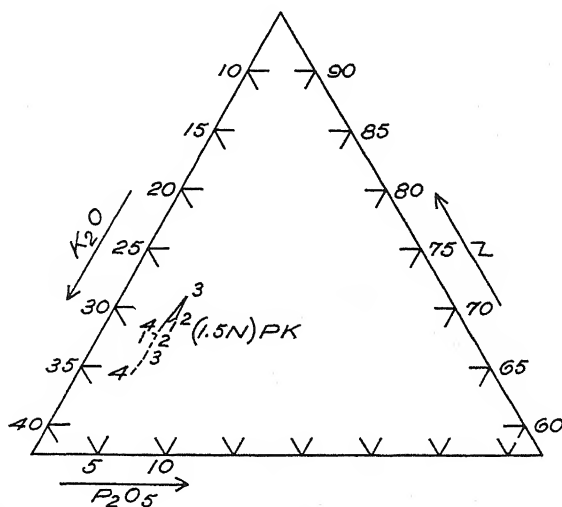


FIG. 4. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [(1.5N)PK].

From the manner in which the *NPK-units* are derived (6) one would expect that the relative changes in direction of an element in the *NPK-unit* would be identical with the changes in the direction of the graphs of that element expressed as a percentage of the dried foliage for the period considered (fig. 1). This is in general the case. The deviations from the rule are found in the nitrogen or phosphorus of the following couplets: NP (1.5K)

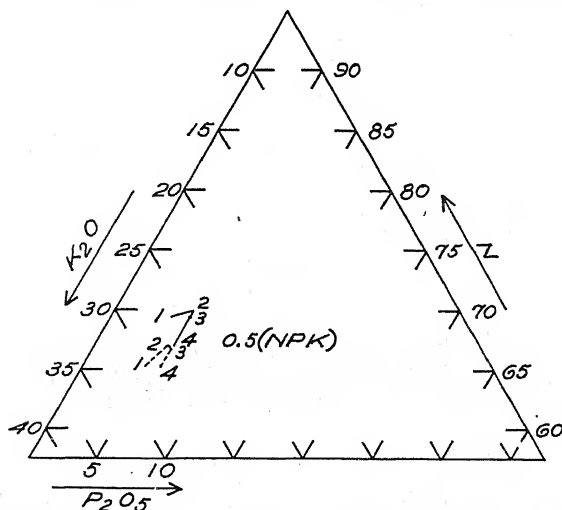


FIG. 5. The relative courses of nutrition of plants on duplicate plots with approximately equal yields, as shown by the *NPK-units* at successive sampling dates [0.5(NPK)].

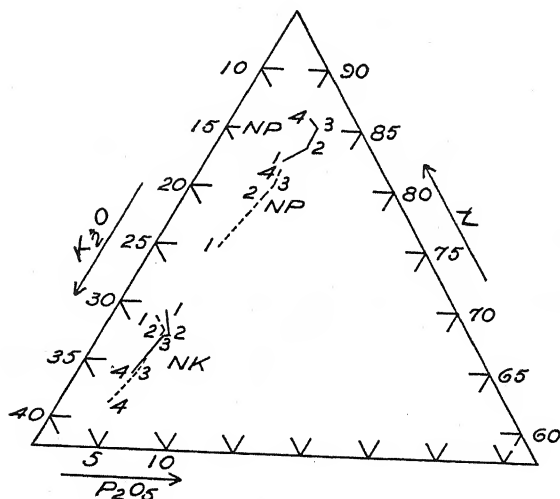


FIG. 6. The relative courses of nutrition of plants on duplicate NP and NK plots respectively; the NK plots differ from each other by less than 10 per cent. in yields, and the NP plots by more than 10 per cent.

(no. 2-12 and no. 5-6), NPK (no. 1-10 and no. 4-8) NK (no. 1-7 and no. 4-11), and NP (no. 2-12 and no. 5-6). These divergences are the result of the relatively large differences in the percentage of potash in the leaves of plants growing on the above duplicate plots.

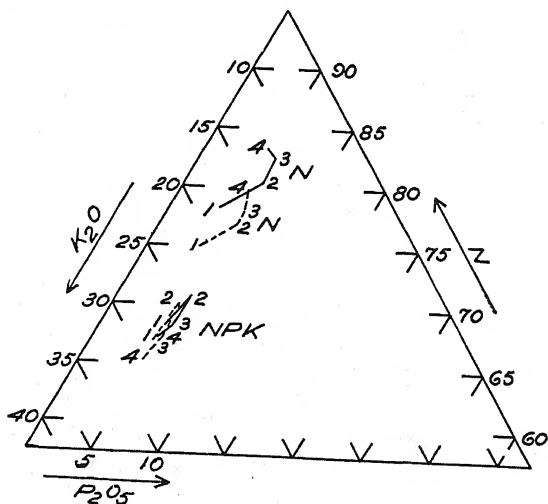
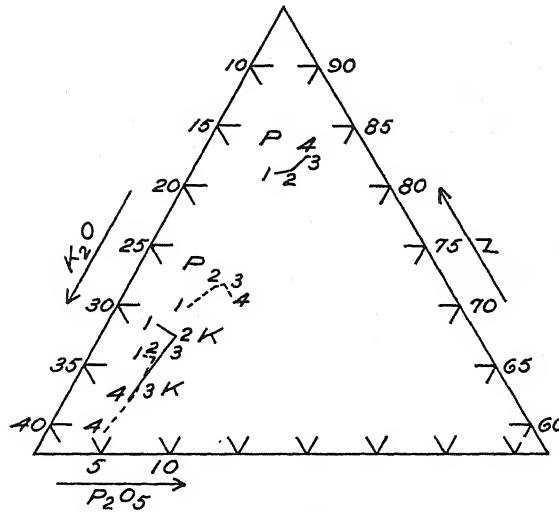


FIG. 7. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective NPK-units on successive sampling dates.



The displacements from one sampling date to another (figs. 2-10) showing the course of nutrition, with respect to nitrogen, phosphoric acid, and potash are rarely identical in the duplicates. In some pairs of duplicates the divergences may be so great that the trilinear coordinate diagrams may be

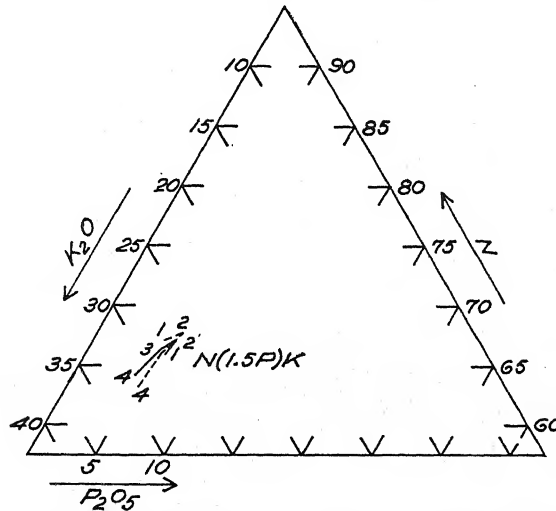


FIG. 9. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates [N(1.5P)K].

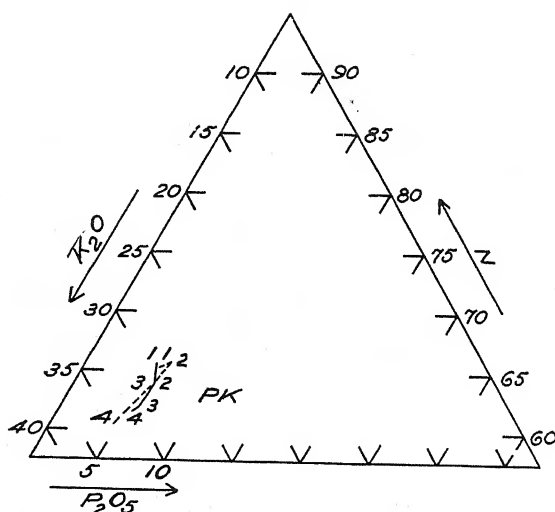


FIG. 10. The relative courses of nutrition of plants on duplicate plots differing by more than 10 per cent. in yield, as shown by their respective *NPK-units* on successive sampling dates [PK].

distinctly separated at all sampling dates [figs. 3, 5, 6 (NP), 7 (N), 8 (P)] and in others the diagrams may overlap and the lines of one member of a pair may intersect or nearly coincide with those of its duplicate [figs. 2, 4, 6 (NK), 7 (NPK), 8 (K), 9, 10]. In the latter, the detailed graphs are not sharply differentiated.

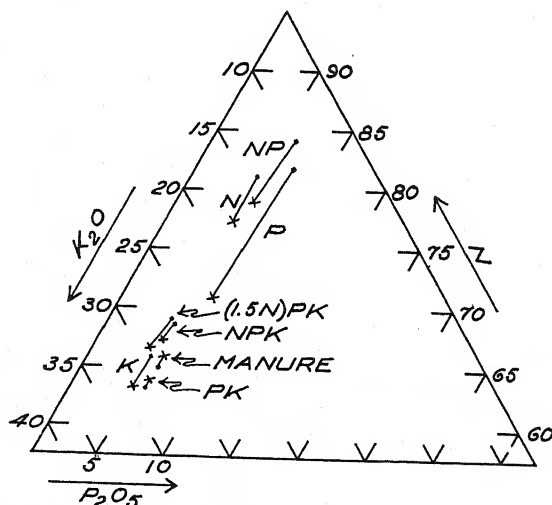


FIG. 11. The relative positions of the mean *NPK-unit* of duplicate plots. The position of the duplicate in tier 4 or tier 5 is indicated by a cross (x).

RELATION OF THE MEAN NPK-UNITS OF SIMILARLY TREATED COUPLETS. —The indications given by the trilinear coordinate diagrams can be analyzed more readily by an examination of figures 11 and 12 which show the mean *NPK-units* in such a way that the points representing the mean *NPK-units* of duplicate pairs of treatments are joined by a straight line. A cross (×) indicates the mean *NPK-unit* of the corresponding plot in tiers 4 and 5. The mean *NPK-unit* corresponds to the center of gravity of the respective diagrams in figures 2–10.

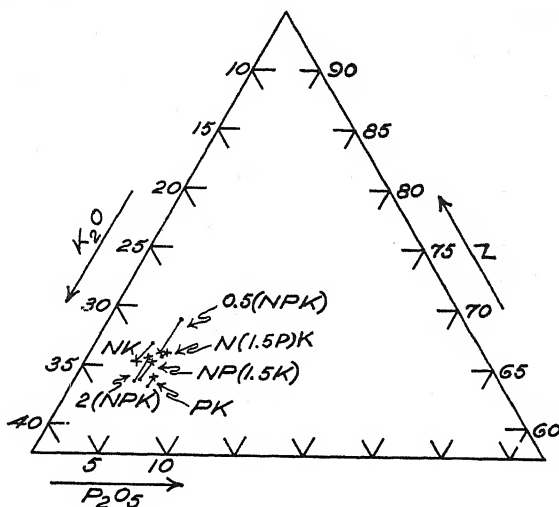


FIG. 12. The relative positions of the mean *NPK-units* of duplicate plots. The position of the duplicate in tier 4 or in tier 5 is shown by a cross (×).

The differences in the displacements of the mean *NPK-units* (figs. 11 and 12 and table I) between duplicate pairs are relatively less for certain heavily fertilized plots [manure, 2(NPK), N(1.5P)K, NP(1.5K)] than for plots with relatively lower fertilization (N, NP, P). The displacement of the duplicate mean *NPK-unit* toward the left base apex, indicating higher  $K_2O$  and lower N in the *NPK-unit*, occurs with higher yield in 2(NPK), 0.5(NPK), manure, NK, NP(1.5K), and NPK, all of which have yields above 150 pounds, and all differ less than 10 per cent. between duplicates; NP, of which the duplicates differ by 12.9 per cent., also has relatively higher  $K_2O$  in the mean *NPK-unit* of the higher-yielding plot. On the other hand, the displacement of the *NPK-unit* is toward the vertical apex (lower  $K_2O$  and higher N) for the duplicate with higher yield in N, P, K, PK, and (1.5N)PK, all except the last of which differ by more than 12.9 per cent. between the yields of duplicate plots. With respect to the relative composition of the mean *NPK-unit*, therefore, the yields of duplicate N, P, K, and PK plots are anomalous.



Displacements of duplicate *NPK-units* with respect to the right base apex show no consistent relations with relative yield.

Accompanying these differences in the  $\text{N-P}_2\text{O}_5\text{-K}_2\text{O}$  equilibrium (*i.e.*, the *NPK-unit*) between duplicates, the differences in the *intensities of nutrition* of the respective duplicates may be great, medium, or small. In the group of seven plots showing small relative differences in yields between duplicates [2(NPK, 0.5(NPK), (1.5N)PK, NK, manure, NP(1.5K) and NP] a higher *intensity of nutrition* is accompanied by higher yields, except in one case—*viz.*, (1.5N)PK (no. 2-4 and no. 5-14). In the group of six plots showing large differences in yields of duplicates [N, N(1.5P)K, NPK, P, PK, and K] a higher *intensity of nutrition* is accompanied by higher yields in three cases and by lower yields in the other three; the latter are N, P, and PK, which as indicated above are anomalous in other respects.

There are, accordingly, nine out of the thirteen pairs of duplicates in which a higher *intensity* is accompanied by higher yields. In seven of these nine cases, the higher *intensity* is the result of an increase in the potash of the *NPK-unit* made at the expense of the nitrogen. In the other two cases—*viz.*, K(no. 1-4 and no. 4-14) and N(1.5P)K (no. 2-8 and no. 5-10)—the higher *intensity* of the higher yielding duplicate is the result of higher nitrogen in the *NPK-unit* made at the expense of the phosphoric acid and potash.

The four pairs of duplicates in which higher *intensity of nutrition* is accompanied by lower yields have abnormal positions on the triangle. In one pair of duplicates—*viz.*, (1.5N)PK (no. 2-4 and no. 5-14)—the lowest yielding plot (no. 5-14) has a very low phosphoric acid content in the *NPK-unit* and lies further away from the apex of the triangle where  $\text{P}_2\text{O}_5 = 100$  per cent. than in any of the plots except the NK plot (no. 4-11). Three pairs of duplicates—*viz.*, N(no. 1-2 and no. 4-16), P(no. 1-3 and no. 4-15), and NP(no. 1-6 and no. 4-12)—are situated much higher on the triangle towards the apex  $\text{N} = 100$  per cent. than the other plots.

The relations of the foliar diagnosis of the N duplicates (no. 1-2 and no. 4-16) and also of the P duplicates (no. 1-3 and no. 4-15) are anomalous, as has been shown, both with respect to the *intensities* and the *NPK-units*. In these plots both the *intensity of nutrition* and the *NPK-units* of the higher yielding duplicate is further removed from the optimum *intensity* and the optimum *NPK-unit*.

The evidence derived from more recent work in the Jordan fertility plots of this Agricultural Experiment Station is that this abnormality is the result of differences in calcium, the effect of which is to reduce the potash and increase the nitrogen and phosphoric acid in the *NPK-unit*.

Because of the topography of plots no. 4-14 (K), no. 4-15 (P), and no. 4-16 (N), some of the subsurface clay is exposed by erosion. The pronounced acidic functions of clay apparently have caused factors other than the ones

varied (nitrogen, phosphoric acid, and potash) to produce the differences in yields.

As already pointed out (6), plot experiments are based on the assumption that all the other factors are equal except those which are varied. If the calcium or magnesium or any other factor is greatly different in two plots, the foliar diagnosis with respect to N,  $P_2O_5$ , and  $K_2O$  might be inadequate to explain the differences in yield. Calcium and magnesium could not be determined in these experiments because of the use of calcium-containing sprays. The foliar diagnosis of potato plants sprayed with non-calcium-containing sprays is now under investigation.

The PK duplicates (no. 1-8 and no. 4-10) differ by over 40 per cent. and yet the mean *intensities of nutrition* and the mean *NPK-units* differ but little. Differences that exist in nutrition, slight as they are, have been shown to be related to differences in yield in a manner inconsistent with that found in other plots. One must, therefore, assume also in the case of these two duplicates the existence of a factor or factors other than N,  $P_2O_5$ , or  $K_2O$  which is the cause of the differences in yield.

Considering all tiers, the highest yields have been obtained with *NPK-units* within limits of N: $P_2O_5$ : $K_2O$  = 63.9-67.4:5.0-5.7:27.7-30.3 and *intensities* within limits of 9.5-10.3. Departure from either one or more of these limits has resulted in yields of less than 195 pounds per 1/100-acre plot.

### Summary

1. The foliar diagnoses of similarly fertilized duplicate pairs of plots from a long-continued field experiment with potatoes were compared. The fertilizer treatments consisted of a single element, combinations of two elements, combinations of all three elements differently equilibrated, and also a manure treatment.

2. Soil heterogeneity is indicated by the relative yields of tubers from duplicate plots. The differences in yields between similarly treated couplets varied from 4.7 per cent. to 54.8 per cent. The divergences were not the result of a factor operating uniformly over the whole area.

3. The differences in the course of nutrition with respect to nitrogen, phosphorus, and potassium resulting from the several fertilizer treatments are shown graphically.

4. The magnitude of the displacements of the *NPK-units* (N- $P_2O_5$ - $K_2O$  equilibrium) from one sampling date to another is never quite identical in the duplicates, although the directions of the displacements are similar.

5. The meteorological factors have not acted identically on all treatments.

6. The relative positions of the mean *NPK-unit* of couplets indicate that in six high-yielding couplets [manure, 2(NPK), N(1.5P)K, NP(1.5K), NK, and NP] differing in yield by 12 per cent. or less, the higher yielding duplicate

has higher  $K_2O$  and lower N in the *NPK-unit* and a higher *intensity of nutrition*. In four low-yielding duplicates having large differences in yields between duplicates (N, P, K, PK) the higher yielding duplicate has higher N and lower  $K_2O$  in the *NPK-unit* with lower *intensity of nutrition* (P) or with intensities nearly identical (N, K, PK).

7. The four couplets in which higher *intensity of nutrition* is accompanied by lower yields have abnormal positions on the triangle. The abnormalities are believed to be the result of differences in the available Ca and Mg of the duplicate plots. Work is under way to test this hypothesis.

THE LABORATORY OF PLANT NUTRITION

DEPARTMENT OF HORTICULTURE

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THE RELATION OF CABBAGE HARDINESS TO BOUND WATER,  
UNFROZEN WATER, AND CELL CONTRACTION  
WHEN FROZEN<sup>1</sup>

J. LEVITT

(WITH ONE FIGURE)

Several investigators have attempted to correlate bound water with hardness in cabbage. ROSA (18) was the first to discuss the point. He measured total ice in hardened and unhardened plants by means of the dilatometer. Greater quantities of water remained unfrozen in the former, even though total moisture content was less. He further found that hardened tissue dried more slowly at 60° C. than did tender tissue. BOSWELL (1) confirmed this. But ROSA's results, however suggestive, tell nothing about colloiddally bound water. At the relatively high temperatures he used (mainly -5° C.) most of the unfrozen water must have been held by osmotic forces, and the difference between hardened and unhardened was at least mainly due to the greater concentration of the former's sap. The oven-drying results may quite readily be explained by a less water-permeable cuticle in the hardened, as indeed the more waxy appearance seems to indicate is the case.

KIMBALL (11), using NEWTON and GORTNER's cryoscopic method, found that the percentage of bound water usually varies inversely with hardness.

GREATHOUSE (5), with the same method, obtained a direct correlation between bound water and hardness. Statistical analysis of his results led him to conclude that bound water and osmotic pressure are not closely correlated, and that hardness is more closely correlated with the former than with the latter. Nonetheless, it is highly probable that the increase in bound water is at least partly due to that bound by sugars, which may be present in larger quantities even in plants with lower total osmotic pressure. Furthermore, table I shows that when calculated per gm. of dry matter, the amount was not any greater in the hardened than in the unhardened.

DUNN (3) determined the dye adsorption of cabbage juice and took this as a measure of the relative quantity of hydrophilic colloids, presumably on the assumption that dye adsorption and water adsorption are correlated. The average amount of dye adsorbed was higher in the hardened than in the unhardened. Recently (4) he has concluded that the method is not very reliable as a measure of hardness.

LEVITT and SCARTH (13), using a plasmometric method, were unable to detect any non-osmotically bound water in either hardened or unhardened

<sup>1</sup> Investigation carried out at the Division of Plant Physiology, University of Minnesota, under a Royal Society of Canada Fellowship.

TABLE I

BOUND WATER OF UNHARDENED AND HARDENED CABBAGE (VARIETY, EARLY JERSEY  
WAKEFIELD). ADAPTED FROM GREATHOUSE

TREATMENT	CONCENTRATION BY REFRACTOMETER	BOUND WATER	BOUND WATER PER GM. DRY MATTER
	%	%	gm.
15% soil moisture .....	8.2	6.53	0.80
14% " " .....	8.4	7.90	0.94
Periodically wilted .....	11.0	9.53	0.87

cabbage. There are two objections to these results. The lack of sensitivity of the method may conceivably fail to reveal a binding of 2-3 per cent. of the total water; and since the determinations were made at room temperature, the hydration would be considerably less than that obtained by the other methods (except the dye adsorption test).

Of the numerous methods used for measuring bound water, the consensus of opinion now seems to favor the calorimetric (SAYRE 19, MEYER 15, etc.). It has one outstanding advantage which is usually overlooked—the material is tested in the normal, living condition, and therefore gives a measure of total bound water. There is no loss due to coagulation of the proteins as is the case with any procedure which kills the tissues (*e.g.* the cryoscopic and probably the dilatometric.). Furthermore, it is amenable to at least as high a degree of accuracy as any, and is free from complications such as arise when foreign solutes are added as in the cryoscopic method.

However, as heretofore used, the calorimetric method (as well as the dilatometric) measures total bound water—that held colloiddally, osmotically, and by hydration of sugars. Consequently, differences in quantity may be due to any or all of these three. DEXTER (2), in fact, found that after standing for 40 hours in 10 per cent. sucrose, 2.5 per cent. sucrose, and water respectively, cabbage leaves contained quantities of bound water in that order.

In the following investigation a modification was introduced which enabled the separate calculation of osmotically and non-osmotically bound water. LEBEDINCEV (12) made use of a similar correction for the dilatometric method.

### Methods and results

MÜLLER-THURGAU (16) first evolved the calorimetric technique for determining the quantity of ice in frozen plant material. His technique is now regarded as too crude, though his results are probably sufficiently accu-

rate, since he compensated for lack of sensitivity in his apparatus by use of large samples (100 gm.).

The technique employed naturally depends on the kind and quantity of material used. MEYER (15) filled metal, rubber-stoppered tubes with 20-gm. samples of pine needles and froze these by immersion in a salt solution at the required temperature. SAYRE'S (19) method is essentially the same. When freezing was complete, the tube was removed, unstoppered, and the needles prodded out into the calorimeter.

This was found unsuitable for cabbage, mainly on account of its high moisture content. Some water was always lost by condensation on the inside of the tube. There was sometimes a tendency for the material to stick to the walls and this increased the time required for transfer, which even under the best conditions is long enough to allow a temperature change.

ROBINSON (17) using much smaller samples (0.4 to 0.8 gm.) wrapped the material in tinfoil, which was placed in a weighing bottle and frozen in air. Transfer to the calorimeter—tinfoil and all—was in this way accomplished with maximum rapidity.

When using large samples, however, this method has its disadvantages. In the case of water or solutions, the tinfoil is burst due to the expansion, and there may be some loss of material. At the high freezing temperatures used in the following investigation, enclosure of the wrapped sample in a weighing bottle was found very favorable to undercooling. Because of these difficulties, a new type of container was adopted.

A cylindrical cup (A, fig. 1), 4 inches long by 0.75 inch in diameter, together with a close-fitting cap are constructed from sheet brass (0.02" to 0.035" shimstock). The lid has a flanged edge which allows it to slip easily over the cup, and yet when squeezed in place it makes a water-tight contact with a collar near the top of the cup. Before the container is soldered together all the pieces are weighed. This allows an exact calculation of the specific heat of the finished container since those of the brass and the solder are known (0.09 and 0.045 respectively). The capacity is about 25 cc.

The advantages of these containers are several. They are unbreakable. They are good conductors of heat and therefore allow temperature equilibria to be reached in minimum time. Their specific heat is small and is exactly known. They are water-tight and therefore do not require enclosure in a weighing bottle which would greatly favor undercooling. (When filled with a sample and left in the chamber at 0° C. with the full force of the fan on them for 24 hours, they lost not more than 1 mg. in weight.) When used correctly (see below) they are never burst by ice formation. Their transfer from the chamber to the calorimeter is instantaneous and therefore no appreciable temperature change occurs.

The samples were frozen in a cold chamber equipped with a fan and thermoregulator. The temperature fluctuated not more than  $\pm 0.4^{\circ}$  C. This range of variation was narrowed by use of a double-walled can retained inside the chamber. The space between the two walls was filled with salt solution, and the inner can was covered with a lid perforated to accommodate a thermometer. The temperature of the air in the inner can kept constant to  $\pm 0.1^{\circ}$  C. A pair of tongs was kept in the chamber, with which to handle the samples and the lid of the can.

Even pure water may remain unfrozen for 12 or more hours at a few degrees below the freezing point. Supercooling was prevented by maintaining the cups slightly agitated, in the following way. A square of wire screen was tied to a metal ring on a retort stand and a cardboard "sail" suspended below the screen. The force of the fan on this sail was sufficient to keep the screen (together with the samples placed on it) in constant

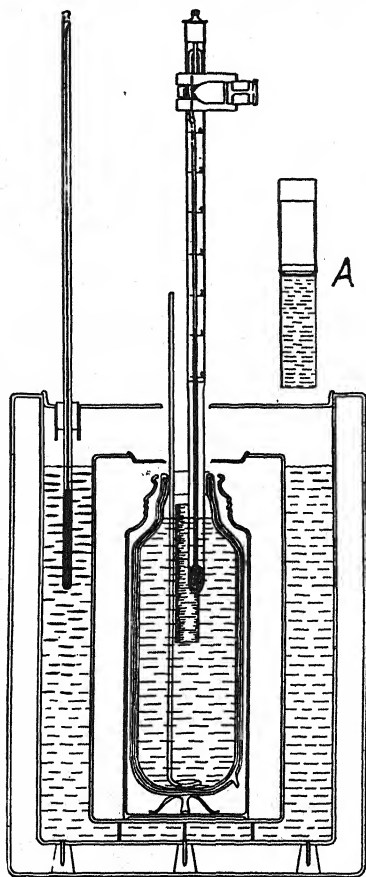


FIG. 1. Design of cup (A) and calorimeter used in the determinations.

gentle motion. When frozen, the samples were transferred from the screen to the can and left for two or three hours to come to equilibrium.

A pint thermos bottle fitted with a stopper, Beckman thermometer, and stirring rod served as the calorimeter (fig. 1). Into it were pipetted 400 cc. of distilled water at a standard temperature (in this investigation  $21.4 \pm 0.2^\circ \text{C.}$ ). Since even a thermos bottle is not perfectly insulated, it was necessary to retain it in an atmosphere of as nearly constant temperature as possible. This was accomplished by means of a triple-walled can. Between the outer two walls was a dead-air space, while water was retained between the inner two. By adjusting the temperature of the water, the air in the innermost can (which contained the thermos bottle) was readily maintained at a constant temperature ( $0.9^\circ \pm 0.15^\circ \text{C.}$  above the equilibrium temperature attained by the calorimeter after the frozen sample had been added). The sample used was such as to cause a  $2.5^\circ \text{C.}$  ( $\pm 0.15^\circ \text{C.}$ ) drop in the temperature of the calorimeter. All the thermometers in use were calibrated against a precision thermometer.

#### CALIBRATION OF CALORIMETER

About 10 gm. of water were accurately weighed in the tared containers. The cups were then laid on their sides on the metal screen inside the cold chamber. (If stood up, the cups either burst or the back pressure of the cup walls prevents complete freezing.) After 12 or more hours, they were transferred to the double-walled can inside the cold chamber and allowed a further three hours to come to equilibrium.

The calorimeter itself was set up at the same time and allowed three hours to come to equilibrium with the air in the constant temperature can. Its temperature was then read and a cup quickly transferred to it. After half an hour, stirring was commenced and continued until the temperature became constant (5 to 10 min.). The end point was readily distinguished as it remained constant (to  $1/400^\circ \text{C.}$ ) for 10 or more minutes.

For each following determination the calorimeter must be emptied, wiped dry, and refilled with water at the required temperature. It is then allowed about 10 min. to reach equilibrium.

The calibration factor ( $F$ ) is calculated from the following formula (using MEYER'S symbols):

$$F = \frac{W_w S_i T_s + W_w S_w T_e + W_c S_c (T_e + T_s) + W_w Q}{NS_w (T - T_e)}$$

where  $W_w$  = wt. water

$W_c$  = " cup

$S_i$  = sp. heat ice (0.50—see SAYRE)

$S_w$  = " " water (1.004—see SAYRE)

$S_c$  = " " cup (0.08–0.085)



$T$  = temp. of calorimeter at start  
 $T_e$  = " " " " end  
 $T_s$  = " " sample (*i.e.* + °C. below 0)  
 $Q$  = Heat of fusion of ice in calories (79.71)  
 $N$  = Vol. water in calorimeter (cc.)

The results were as follows:

TABLE II

CALIBRATION OF CALORIMETER WITH WATER FROZEN AT -5.6° C.

$W_c$	$W_w$	TIME FROZEN	$T-T_e$	$T_e$	TEMPERATURE OF CAN	FACTOR
<i>gm.</i>	<i>gm.</i>	<i>hr.</i>	<i>°C.</i>	<i>°C.</i>	<i>°C.</i>	
6.932	9.821	18	2.4175	18.6725	19.6	1.039
8.285	9.799	19	2.425	18.97	19.75-19.8	1.040
7.125	9.8175	20	2.4275	19.095	19.9 -19.95	1.039
8.640	9.856	21	2.4475	18.89	19.9	1.036
					Av. ....	1.0385 ± .002

#### DETERMINATIONS WITH PURE SOLUTIONS

As a test of the method, measurements were first made in the complete absence of colloiddally bound water. The amount of ice formed was compared with the quantity that should arise as estimated from the freezing point ( $T_m$ ) of the solution ( $W_f = W_w - \frac{T_m}{T_s} \times W_w$ ). A modification of MEYER'S formula for free water was used to avoid the necessity of knowing the value of  $Q$  at the freezing point. The ice is assumed to thaw at 0 °C. (HAMPTON and MENNIE [8]). This gives exactly the same result as MEYER'S formula and it has the advantage of simplicity.

$$W_f = \frac{FNS_w (T - T_e) - [(T_e + T_s) (W_d S_d + W_w S_w + W_c S_c)]}{Q - 0.5T_s}$$

where  $W_d$  = wt. of dry matter  
 $S_d$  = sp. heat of dry matter  
 $S_b$  = sp. heat of bound water (assumed =  $S_w$ )

Other symbols as in the previous formula.

These results indicate about 5 mols water bound per mol sucrose (table III).

According to SCATCHARD (21), sucrose is hydrated probably with six molecules of water, and a solution is a mixture of water, anhydrous sucrose, and hydrated sucrose. His figures for the average amount bound at 0° C. range between 6 molecules for the most dilute and 3.8 molecules for the most concentrated solutions. Since the concentration of the solution in table III was between these two extremes, the value of five molecules of water per molecule of sucrose agrees well with SCATCHARD'S calculations.

TABLE III

 $\frac{M}{2}$  SUCROSE FROZEN AT  $-5.6^{\circ}$  C.

FROZEN	$W_w$	$W_d$	$W_c$	$T-T_o$	$T_o$	$T_m$	$W_f$	$W_b$	$\frac{W_b}{W_d}$
<i>hr.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$		<i>gm.</i>	<i>gm.</i>	
20	12.725	2.185	8.503	2.65	18.73	1.025	9.891	0.619	0.283
21	12.717	2.184	7.121	2.6375	18.7525		9.8695	0.637	0.292
22	12.746	2.189	6.929	2.665	18.88		9.991	0.516	0.236
23	12.697	2.180	8.277	2.6575	18.7675		9.940	0.531	0.244
								Av. ...	0.264

## DETERMINATIONS WITH CABBAGE SEEDLINGS

Before applying the method to measure bound water in cabbage seedlings it is first necessary to find the specific heat of the dry matter which constitutes the tissues. This, however, involves difficulties, for it is essential that the same standard conditions prevail (including the  $2.5^{\circ}$  temperature drop of the calorimeter). The following method was finally adopted:

New cups were made with dimensions similar to the above-described standard cups, save that they were hemispherical in cross section and fitted inside these. After filling with weighed quantities of dried cabbage tissue the hemi-cups were sealed (by soldering) and placed inside the standard cups. Enough water (about 9.3 cc.) was now pipetted into the standard cups to enable the whole when frozen to cause the usual  $2.5^{\circ}$  C. drop in the temperature of the calorimeter. The specific heat of the hemi-cups was 0.07. It is essential that the hemi-cups be perfectly sealed, otherwise the water penetrates and invalidates the results.

In order to calculate the non-osmotically bound water, one must know the total moisture and the freezing point of the tissues. To eliminate the

TABLE IV

SPECIFIC HEAT DETERMINATIONS WITH DRIED CABBAGE PETIOLES AT  $-5.6^{\circ}$  C.

$W_c$	$W_t$ HEMI.- CUP	$W_w$	$W_d$	$T-T_o$	$T_o$	SPECIFIC HEAT
<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	$^{\circ}\text{C.}$	$^{\circ}\text{C.}$	
8.2815	11.5207	9.1625	6.4245	2.415	18.995	0.251
6.929	10.6095	9.1575	6.451	2.40	18.975	0.242
8.2815	11.5207	9.166	6.4245	2.4225	18.9025	0.275
7.122	11.5207	9.159	6.4245	2.4075	18.8225	0.263
8.466	10.6095	9.186	6.451	2.41	18.8625	0.237
					Av. ....	$0.254 \pm .02$

error of sampling all three tests were made on one and the same sample in the following way:

After the calorimetric determinations, the cups were transferred to a vacuum oven and dried to constant weight at 60° C. (to prevent caramelization which would occur at 100° C.). Sufficient water was then added to restore the original weight of the fresh tissue. After storing for two days at +2° C., to enable the dried tissue to soak up the water, the juice was expressed and the freezing point determined. Thus total moisture, total ice, and freezing point were all determined on the same sample.

An objection to this method of determining the freezing point is that a certain amount of hydrolysis of disaccharides may occur during the drying. However, since most of the sugars present in cabbage are monosaccharides (HARVEY, 9) the error cannot be very great. An idea of the extent of error was obtained by comparing the freezing point obtained in this way with that of a duplicate sample whose juice was expressed and tested immediately after thawing. Both sets had been frozen 24 hours at -8° C., a temperature sufficient to kill the tissues.

	VACUUM DRIED	CONTROL
Unhardened .....	-0.94 °C.	-0.92 °C.
“ .....	-0.88	-0.81
Hardened .....	-1.35	-1.31
“ .....	-1.12	-1.14

Judging from these figures, the error introduced is slight, probably less than the variation between samples.

Knowing  $W_t$  at temperature  $T$  and subtracting this from total water, the result obtained is the total “bound water” at this temperature—osmotic plus non-osmotic. Separate determinations of these two constituents are possible on the prevalent assumption that the non-osmotically bound water is that portion which does not act as a solvent, *i.e.*, which does not obey the laws for ideal solutions.

If  $W_w$  = total water

$W_f$  = frozen water

$W_o$  = osmotically bound water

$W_b$  = non-osmotically bound water

Then  $W_w = W_f + W_o + W_b$

But  $W_o = \frac{T_m}{T_s} (W_w - W_b)$

Therefore  $W_b = W_w - \frac{W_f}{1 - \frac{T_m}{T_s}}$

These quantities were determined for unhardened and hardened (2 weeks at +5° C. with continuous light) cabbage plants when  $T = 5.6^\circ$  C. below zero (table V). Only the petioles were used as these pack easily into the cups. Each sample contained the petioles of about a dozen plants.

TABLE V  
UNHARDENED CABBAGE FROZEN AT  $-5.6^{\circ}\text{C}$ .

TIME FROZEN	MOIS- TURE	$W_w$	$W_d$	$W_e$	$T - T_e$	$T_e$	$T_m$	$W_f$	$\frac{W_f}{W_d}$	$\frac{W_f}{W_w + W_d}$	$\frac{W_w - W_f}{W_d}$	$W_b$	$\frac{W_b}{W_d}$
hr.	%	gm.	gm.	gm.	$^{\circ}\text{C}$ .	$^{\circ}\text{C}$ .		gm.				gm.	
15	92.0	12.376	1.076	6.181	2.7225	18.89	0.80	10.563	9.84	0.79	1.68	0.051	
26	93.0	12.201	0.918	6.685	2.695	18.565	0.802	10.523	11.50	0.80	1.83	-0.087	
38	92.2	12.176	1.028	6.181	2.6525	18.3575	0.842	10.340	10.09	0.79	1.78	0.011	
23	91.3	11.635	1.102	8.277	2.585	19.2375	0.72	9.927	9.03	0.78	1.55	0.238	
25	91.8	11.668	1.044	6.929	2.6125	19.0675	0.68	10.146	9.75	0.80	1.46	0.120	
21	93.3	11.638	0.840	6.181	2.6075	18.9325	0.715	10.186	12.16	0.82	1.73	-0.057	
22	92.2	11.502	0.991	6.685	2.585	19.305	0.715	10.023	10.14	0.80	1.49	0.008	
23	92.5	11.592	0.946	8.635	2.6125	19.16	0.725	10.106	10.71	0.81	1.57	-0.024	
								Av. ....	10.40	0.80	1.64	0.032	0.03

HARDENED CABBAGE FROZEN AT $-5.6^{\circ}\text{C}$ .													
16	89.0	12.759	1.577	8.463	2.63	18.98	1.23	9.810	6.24	0.69	1.87	0.189	
25	88.0	12.600	1.724	8.463	2.56	19.52	1.287	9.371	5.45	0.66	1.87	0.430	
27	86.8	12.455	1.895	8.503	2.4675	18.475	1.392	9.091	4.81	0.63	1.77	0.356	
22	87.6	12.483	1.762	8.463	2.5825	18.9225	1.16	9.684	5.51	0.68	1.59	0.271	
24	87.2	12.394	1.826	7.121	2.5275	19.2375	1.215	9.350	5.14	0.66	1.67	0.453	
								Av. ....	5.43	0.66	1.75	0.340	0.20

The most striking difference between the two sets of cabbages is in the quantity of ice formed, almost twice as much per gm. dry matter ( $\frac{W_f}{W_d}$ ) in the unhardened as in the hardened (10.40 and 5.43 gm., respectively). This is obviously due to the difference in the total moisture and the freezing point, both of which are lower in the hardened.

Expressed on the basis of fresh weight, ( $\frac{W_f}{W_w + W_d}$ ), the non-hardened contained 0.80 gm. ice per gm. of tissue, while the hardened had only 0.66 gm. This means that if the ice formed extracellularly, the unhardened cell was reduced to one-fifth its original volume, the hardened only to one-third.

The total unfrozen water per gm. dry matter, ( $\frac{W_w - W_f}{W_d}$ ), is slightly greater in the hardened than in the unhardened (1.75 and 1.64 respectively).

The non-osmotically bound water has a very small value in both cases, though considerably larger in the hardened plants—0.20 gm. per gm. of dry matter as compared to 0.03 gm. in the unhardened.

MEASUREMENTS AT CRITICAL FREEZING TEMPERATURES.—In order to apply results of this type to frost resistance it is essential to obtain measurements of the above quantities not at a random freezing temperature but at the two points just low enough to cause the same degree of injury (*e.g.* 50–75 per cent.) in hardened and unhardened plants respectively, *viz.*, at the critical freezing temperature of each.

The results already recorded in table V were obtained at the critical freezing temperature for hardened plants. Table VI shows the injury that

TABLE VI  
INJURY TO HARDENED CABBAGE FROZEN AT  $-5.6^{\circ}\text{C}$ . EACH VALUE AN  
AVERAGE OF NINE PLANTS

GROUP	TIME FROZEN	INJURY
	<i>hr.</i>	%
1	9	55
2	16	45
3	20	50

resulted. Unhardened plants, on the other hand, suffer as much at considerably higher temperatures (table VII). It should be emphasized that the plants tested for hardiness were in all cases taken from the same group and frozen at the same time as those tested calorimetrically.

When calorimetric determinations were made at  $-5.6^{\circ}\text{C}$ ., control unhardened plants were simultaneously tested for resistance to this temperature, always with the expected result, 100 per cent. injury.

TABLE VII

RESISTANCE OF UNHARDENED CABBAGE SEEDLINGS. EACH VALUE AN AVERAGE OF EIGHT PLANTS

GROUP	TEMPERATURE	TIME FROZEN	INJURY
	°C.	hr.	%
1	-2.7	10	100
2	-2.1	11	85
3	-1.4	16	25

The critical temperature for the unhardened is apparently about  $-1.8^{\circ}\text{C}$ . The calorimetric determinations were made at  $-2.1^{\circ}\text{C}$ . (table VIII). At this relatively high temperature it was found necessary to inoculate the cups of petioles with an ice crystal in order to insure freezing. The potted plants, however, froze readily, apparently on account of their thin leaves, which were in constant gentle motion due to the force of the fan.

A comparison between these values for unhardened plants at their critical freezing temperature ( $-2.1^{\circ}\text{C}$ .) and those already obtained for hardened plants at theirs ( $-5.6^{\circ}\text{C}$ .) yields several important facts.

First, although the amount of ice formed per gm. of dry matter is about 75 per cent. greater in the unhardened than in the hardened cells (9.43 and 5.43 gm. respectively), the former retain 3.5 times as much water in the liquid state as do the latter (6.30 and 1.75 gm. per gm. of dry matter respectively). Even on the basis of fresh weight (though this is not shown in the table) there is twice as much water per gm. in the unhardened as in the hardened (0.40 and 0.21 gm. respectively). There can, therefore, be no question of a reduced degree of dehydration causing the increased frost resistance.

Second, the unhardened contain only 0.56 gm. ice per gm. of fresh weight though suffering 85 per cent. injury, while the hardened have 0.66 gm. and show only 50 per cent. injury. This means that if the ice formed extracellularly, most of the unhardened cells are killed when contracted roughly to half their original volume, the hardened cells only after a shrinkage to one-third. (The greater specific gravity of the hardened will only tend to enhance this relationship.)

Finally the quantity of non-osmotically bound water per gm. of dry matter is greater in the unhardened than in the hardened (0.78 and 0.20 gm.).

It is important to establish whether the injury really is caused by extracellular ice formation as assumed above. Two other possibilities exist: the damage may occur as a result of intracellular ice formation or it may arise during thawing. Since in the above tests both freezing and thawing were rapid, the effect of slowing up these processes was next investigated.

TABLE VIII

UNHARDENED CABBAGE FROZEN AT  $-2.1^{\circ}\text{C}$ .

TIME FROZEN	MOISTURE	$W_w$	$W_d$	$W_c$	$T - T_o$	$T_o$	$T_m$	$W_i$	$\frac{W_i}{W_d}$	$\frac{W_i}{W_w + W_d}$	$\frac{W_w - W_i}{W_d}$	$W_b$
hr.	%	gm.	gm.	gm.	$^{\circ}\text{C}$ .	$^{\circ}\text{C}$ .		gm.				gm.
42	94.1	14.1135	0.879	7.116	2.4475	18.205	0.762	8.448	9.61	0.56	6.46	0.872
43	94.0	14.1095	0.8975	6.173	2.4625	18.6025	0.762	8.475	9.44	0.56	6.28	0.825
44	93.9	14.0985	0.9145	6.680	2.47	18.85	0.805	8.457	9.25	0.56	6.17	0.392
								Av. ...	9.43	0.56	6.30	0.70

Two sets of unhardened cabbages were placed in the chamber at  $-0.5^{\circ}\text{C}$ . and the temperature was gradually and uniformly lowered during a three-hour period to  $-2.1^{\circ}\text{C}$ . (*i.e.*,  $0.5^{\circ}$  per hr.). This necessitated hand regulation, the temperature varying not more than  $\pm 0.2^{\circ}\text{C}$ . Just when ice formation began it is impossible to say, but it was sufficient to be observed at  $-1.5^{\circ}\text{C}$ .

A third set of plants was placed in the chamber at  $-2.1^{\circ}\text{C}$ . Fifteen hours later this fast-frozen set and one of the slow-frozen were removed and immediately thawed at room temperature. The other slow-frozen set was thawed slowly in the cold chamber as follows: the temperature was allowed to rise to  $-1.1^{\circ}\text{C}$ . during 15 min., at which it was retained for one hour. During the next 20 min. it rose to  $+1^{\circ}\text{C}$ ., and 20 min. later the plants were removed from the chamber. In this way thawing occurred during a two-hour period as compared with the others which thawed completely in five minutes.

Table IX shows that neither slow freezing nor slow thawing caused any reduction in injury.

TABLE IX

UNHARDENED CABBAGE FROZEN 15 HOURS AT  $-2.1^{\circ}\text{C}$ . EACH RESULT  
AN AVERAGE OF EIGHT PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	45
Slow	Fast	75
Slow	Slow	80

An older set of unhardened plants, which owing to their stunted growth were sufficiently hardy to have their critical freezing temperature lowered to  $-3.1^{\circ}\text{C}$ . was similarly tested. This time slow freezing was begun with 1 hr. at  $-1.2^{\circ}\text{C}$ ., followed by 1.5 hr. at  $-2.2^{\circ}\text{C}$ . (after a half hour at this temperature freezing was first noticeable). Slow thawing was induced during a three-hour period in which the temperature gradually rose to  $+3.5^{\circ}\text{C}$ . Here again, no protective effect was produced by reducing the rate of freezing or thawing (table X).

TABLE X

UNHARDENED (SLIGHTLY HARDY) CABBAGE FROZEN 8.5 HOURS AT  $-3.1^{\circ}\text{C}$ .  
EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	90
Slow	Fast	65
Slow	Slow	95



Fully hardened plants were next tried. The temperature was dropped  $1^{\circ}$  per hour, from  $-3.3^{\circ}$  C. to  $-7.3^{\circ}$  C. At the end of the first hour almost all were frozen; at the end of the second hour all were frozen stiff. For slow thawing the temperature was raised about  $1^{\circ}$  per hour for 5 hours, at the end of which the temperature was about  $0^{\circ}$  C. and the plants were removed.

TABLE XI

HARDENED CABBAGE FROZEN FOR 15 HOURS AT  $-7.3^{\circ}$  C.  
EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	100
Slow	Fast	80
Slow	Slow	80

The same experiment was repeated on similarly hardened plants at a slightly higher temperature ( $-6.3^{\circ}$  C.). For slow freezing, the temperature was dropped  $1^{\circ}$  C. per hour, from  $-2^{\circ}$  C. to  $-6.3^{\circ}$  C. The first trace of freezing was observable at the end of one hour; after the second hour half were frozen. Slow thawing took 4.5 hours at the end of which the temperature was  $+1^{\circ}$  C.

TABLE XII

HARDENED CABBAGE FROZEN FOR 17 HOURS AT  $-6.3^{\circ}$  C.  
EACH RESULT AN AVERAGE OF NINE PLANTS

RATE OF FREEZING	RATE OF THAWING	INJURY
		%
Fast	Fast	90
Slow	Fast	25
Slow	Slow	15

In both cases the slow freezing reduced the injury somewhat, in neither did the slow thawing have any effect.

Consequently, it appears that under the freezing condition employed, the injury suffered by both the hardened and unhardened plants occurred during freezing and not during thawing. Further, the unhardened were not protected by reducing the rate of ice formation. In the case of the hardened, some protection did result from slowing up the rate of freezing. It seems probable then, that the hardened plants can withstand a cell shrinkage to even less than  $\frac{1}{3}$  of its original volume (which was stated above to be the limit of its endurance).

### Discussion

Before discussing the significance of these figures, it is essential to know how dependable they are. The values for total ice, and the various ways of expressing them, appear quite reliable. On the other hand, the estimation of that portion of the unfrozen water that is non-osmotically bound is open to several sources of error and since the quantity is so small, it can be markedly altered by any of these, a fact which is attested to by the decided variation in the results.

The great difference between this value when determined at  $-5.6^{\circ}\text{C}$ . and at  $-2.1^{\circ}\text{C}$ . for unhardened plants can be due only to error. The slight temperature effect together with the greater but oppositely acting concentration effect can account for only a small fraction of the difference.

One source of error that may explain another portion of this difference is the fact that the freezing point of a solution is not exactly proportional to the concentration, as is assumed in the formula for  $W_b$ .

A slight error in the value for the freezing point of the juice (which as already pointed out probably does occur) may greatly alter the value of  $W_b$ .

Furthermore, the assumption is made that the average specific heat of the dry matter and the water is the same as that of the two when mixed. Here again, the slight error involved may affect  $W_b$ .

It should be emphasized that of all these sources of error, only the last can have any influence (and that a negligible one) on the various values for  $W_t$ .

As a check on the reliability of the values obtained, it is interesting to compare them with those found by other investigators. On the basis of total water, the unhardened contained only 0.3 per cent. at  $-5.6^{\circ}\text{C}$ ., 5 per cent. at  $-2.1^{\circ}\text{C}$ ., while the hardened had 2.7 per cent. at  $-5.6^{\circ}\text{C}$ . These quantities are somewhat lower than those obtained by KIMBALL (11) (mainly 3-6½ per cent.) and by GREATHOUSE (5) (5-9 per cent.) both of whom used the cryoscopic method. However, KIMBALL certainly, and GREATHOUSE probably, used NEWTON and GORTNER's original formula without GROLLMAN's (7) correction (which was approved by GORTNER and GORTNER, 6). This correction always yields lower values than the original formula, and therefore would bring the results of the above workers into closer agreement with our own.

The results show that at any given temperature the hardened plants may have less water withdrawn in the form of ice. But (1) at a temperature which causes practically complete killing of unhardened plants, they retain in their tissues 3.5 times as much water in the unfrozen state as do the hardened at the temperature required to produce the same amount of injury in them; (2) the amount of non-osmotically bound water in hardened plants is very small, and though larger than that for unhardened plants at the same

temperature ( $-5.6^{\circ}\text{C.}$ ), it is nevertheless smaller when the two groups are compared at their respective critical freezing temperatures; (3) at their respective freezing temperatures more ice is formed per gram fresh weight in the hardened than in the unhardened plants.

Cabbage hardness is therefore not determined simply by resistance to dehydration (either of the tissue as a whole, or of the colloidal constituents, *viz.*, the protoplasm in particular), or to the amount of ice which forms, but by some increased ability to resist the effects of freezing.

These facts on the other hand in no way oppose the theory put forward by SCARTH and LEVITT (20). The latter consider that one factor in frost injury is the protoplasmic strain described by ILJIN (10), and that the greater resistance to plasmolysis and deplasmolysis injury which they found in hardened plants is due to a greater resistance to the injurious effects of the strain.

This conception is fully borne out by the fact that cells of hardened plants must be subjected to a contraction to one-third (or even less) of their normal volume before injury sets in, those of unhardened plants to only one-half their normal volume. This may be interpreted as the result of greater resistance to protoplasmic strain in the former, though only to that which occurs during freezing, since rate of thawing has no effect on the degree of injury.

Another hardness factor according to this theory is permeability. Since the rate of freezing is of no detectable importance insofar as unhardened plants are concerned, and since ice forms inside the cell only as a result of rapid freezing, intracellular ice apparently does not arise at the temperatures and the rates of freezing to which they were subjected. Permeability is therefore not the limiting factor in the frost injury of unhardened plants.

Hardened plants, on the other hand, are less injured by slow than by rapid freezing, an indication that intracellular ice is involved, and that the permeability of the hardened cells is insufficient to prevent it. But how does this agree with the fact that hardened cells are more permeable than unhardened (LEVITT and SCARTH, 13)?

Agreement appears as follows: When subjected to its critical freezing temperature the unhardened plant is only  $1\frac{1}{2}^{\circ}$  below its freezing point. Under this small gradient ice formation is relatively slow. The permeability rate (even that of unhardened plants) is sufficient to keep up with it and so to permit extracellular ice formation. In this case, however, protoplasmic strain kills the cells.

On account of its greater resistance to protoplasmic strain, the cell, after hardening, is now able to withstand a lower temperature. But this increases the gradient between the temperature and its freezing point, until at the critical freezing temperature the two are  $4.5^{\circ}\text{C.}$  apart. The gradient is

now more than three times as large as in the case of the unhardened cells when subjected to their critical freezing temperature, and therefore overbalances the greater permeability (which is only about twice that in the unhardened cells (LEVITT and SCARTH, 13). Furthermore, on account of the lower temperature, the permeability is decreased (VAN'T HOFF coefficient is 2.2). It is, therefore, not surprising that the exosmosis of water from the cell is no longer able to keep up with the rapid ice formation, and intracellular ice begins to form.

Instead of resistance to protoplasmic strain being the limiting factor, as when the cabbage cell is in the unhardened state, it is replaced by the other factor—permeability—which is now insufficiently high. With a more gradual temperature drop the situation is reversed. Intracellular ice formation is prevented and a lower temperature is withstood, until the protoplasmic strain (which is now once more the limiting factor) is increased sufficiently to injure the cell.

This may well describe the situation under artificial conditions, where the unhardened plant is suddenly exposed to its critical freezing temperature and the hardened plant to its own, which is  $3.5^{\circ}$  C. lower. In nature, of course, such a difference does not happen. The temperature drop is gradual. How then can the same explanation hold true?

There are two cases in which the plant may behave in essentially the same way as under these artificial conditions. In still air supercooling is considerable, so that even though the temperature drop may be gradual, it is quite possible for ice formation to begin suddenly at several degrees below the freezing point. Also, when the sun sets, or even if suddenly clouded over, the plant which had been thawed by it is now quickly exposed to a temperature considerably below the freezing point. These will afford a similar state of affairs to those provided in artificial freezings.

### Summary

Calorimetric determinations of ice formation in frozen cabbage petioles were combined with determinations of the freezing point and the total moisture. This not only gave a measure of frozen and unfrozen water, but also allowed a separate estimation of osmotically and non-osmotically bound water.

The results were as follows:

#### I. At $-5.6^{\circ}$ C.:

1. A test of the method indicated 5 mols of water bound per mol sucrose in a half molar solution.
2. About twice as much ice per gm. of dry matter occurs in the unhardened as in the hardened cabbages (10.40 and 5.43 gm. respectively).

3. If the ice formed extracellularly, the unhardened cells contracted to one-fifth of their volume, the hardened only to one-third.

4. The hardened plants retained a slightly larger quantity of unfrozen water per gm. of dry matter than did the unhardened (1.75 and 1.64 gm. respectively).

5. The non-osmotically bound water is very small in both, though larger in the hardened than in the unhardened (0.20 and 0.03 gm. per gm. of dry matter respectively).

II. When compared at their critical freezing temperatures ( $-5.6^{\circ}$  C. for hardened,  $-2.1^{\circ}$  C. for unhardened):

1. Unhardened tissue retained 3.5 times as much water (in the liquid state) per gm. of dry matter as did hardened (6.30 and 1.75 gm. respectively). Injury due to dehydration is therefore precluded.

2. The unhardened cell was contracted only to one-half its volume, the hardened to one-third. This indicates the greater resistance of the latter to protoplasmic strain (assuming this to be the cause of injury).

3. The quantity of non-osmotically bound water is greater in the unhardened than in the hardened plants (0.78 and 0.20 gm. per gm. of dry matter respectively).

4. Reducing the rate of freezing and thawing did not alter the critical freezing temperature of the unhardened plants—an indication that injury was due to the cell contraction and not to intracellular ice nor to rapid thawing. With hardened plants, on the other hand, the critical freezing temperature was lowered by reducing the rate of freezing (but not the rate of thawing). This indicates that if intracellular ice-formation was prevented the cells of hardened plants were even more resistant to contraction injury than mentioned above.

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# EFFECT OF POTASH MANURING ON THE PRODUCTION OF ORGANIC MATTER<sup>1</sup>

OSCAR ECKSTEIN

(WITH TEN FIGURES)

## Introduction

The formation of organic matter in the plant is the final result of the manifold chemical reactions and transformations of energy taking place during the course of the growing period. In the study of plant production pot and field experiments afford merely a means of determining the yields of vegetable matter, that is the cumulative effect of the many physiological processes which during the different stages of growth influence the development of the plant to a greater or lesser degree. Furthermore, the results of the usual type of fertilizer experiment only demonstrate the extent to which the amount of organic matter produced by the plant is dependent on the quantity and nature of the plant foods applied and on their joint functioning; but they can tell us little or nothing about the influence of manuring on the various synthetic processes in the plant, such as the formation of carbohydrates or of proteins.

Modern agriculture is not merely concerned with mass production but also aims at achieving a high standard of quality in its products. While this holds good for all branches of agriculture, it is particularly true in the case of modern grassland management and its ultimate products, *viz.*, meat and milk.

The maintenance of yields at a satisfactory level is of course important but the actual value of crops and particularly of the fodder produced, is determined in the main by its composition and more particularly by its content of digestible carbohydrates and proteins. Apart from the fact that reliable methods of estimating resorbable protein are still lacking, the ordinary fertilizer experiment both in the field and in pot cultures fails completely when it is a question of studying the effect of the nutrients applied on the quality of grassland produce.

That it is possible by suitable manurial treatment to influence selectively certain properties of Gramineae—take for example the strengthening of the straw due to potash and phosphoric acid—is a well-known fact (1, 4). On the other hand we have as yet been able to obtain but little insight into the relationship between the effects of fertilizers and the mechanism of plant production in general and the composition of the produce in particular. These relationships can be established more easily, when it is possible to

<sup>1</sup>Read at the Fourth International Grassland Congress, Aberystwyth, Wales, July 13-19, 1937.



obtain an accurate knowledge of the factors favoring the development of the particular property which it is desired to influence (2). It was therefore of interest to ascertain those physiological processes which predominate in determining the quality of the produce and how they can be influenced in the desired manner by manuring.

The most important process in the production of vegetable matter is the assimilation of carbon dioxide by the plant. In addition to making a careful study of the latter, we have also investigated the effect of potash on nitrogen metabolism, transpiration and chlorophyll formation.

All these vital processes are intimately connected with each other and a knowledge of the extent to which they may be influenced by manuring can be obtained only when it is possible to study them in one and the same plant sample under carefully controlled conditions. Moreover, conclusions of practical value can be drawn from the results obtained only when the plants used in such investigations have been grown under completely uniform environmental conditions, *i.e.*, at constant temperature, in uniform illumination, etc. It is obvious that plants grown in the open will not fulfill these requirements; for both in field and in pot experiments it is not possible to grow plants under conditions which remain constant over indefinite periods and can be reproduced at will. Grasses and cereals which are destined to serve as experimental material for comparative and quantitative studies of the influence of manuring on the assimilation of carbon dioxide and other vital processes, must be grown under artificial conditions with a minimum of variation, a requirement the practical fulfilment of which presents considerable technical difficulties. We have, however, been able to meet these requirements to a very considerable extent by the use of the apparatus constructed by GASSNER and GOEZE, a diagram of which is shown in figure 1 (3).

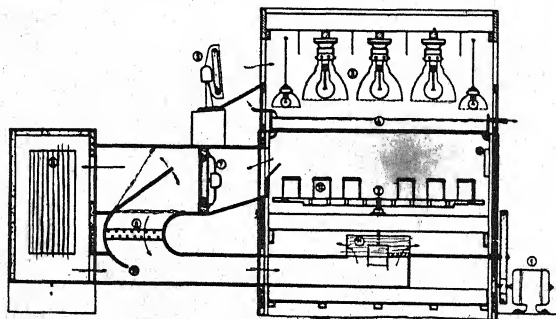


FIG. 1. Diagram of the apparatus.

- |                             |                    |                     |
|-----------------------------|--------------------|---------------------|
| 1. Electric motor           | 5. Oscillating fan | 9. Electric heaters |
| 2. Rotating wooden platform | 6. Thermostat      | 10. Mixing canal    |
| 3. Source of light          | 7. Fans            | 11. Air distributor |
| 4. Shallow tank             | 8. Refrigerator    | 12. Pots for plants |

In designing this apparatus particular care has been taken to insure uniform illumination. This has been achieved by mounting the pots containing the experimental plants on a mechanically-driven (1) rotating wooden disk (2) above which the source of light is situated. The source of light used (3) consists of a battery of Osram-Nitra lamps, some of which are fitted with Zeiss reflectors. A shallow tank with a glass bottom, through which water is kept constantly flowing (4), is interposed between the source of light and the plants and serves to absorb the heat radiated downwards from the lamps while allowing free passage to the light. The lamps themselves are protected from overheating by being constantly exposed to the draught created by an oscillating fan (5).

The temperature of the lower compartment, in which the plants are grown, is kept constant by means of a thermostat (6) and as the air is kept circulating continuously by means of two powerful fans (7), temperature variation is due merely to the slight deviation of the thermostat which, however, does not exceed  $\pm 1^{\circ}$  C. A third fan is connected to the evaporator of a refrigerator (8). Electric heaters (9) are used when higher temperatures are required. Cold and warm air are mixed in the canal (10) and the distributor (11) insures a uniform flow of air into the apparatus, at the same time preventing the current of air from directly striking the pots (12) containing the plants.

As a result of the slight deviation of the thermostat the relative humidity varies by about  $\pm 5$  per cent., while the variation in the  $\text{CO}_2$  content of the air in the apparatus due to penetration of air from without does not exceed  $\pm 5$  per cent. of the  $\text{CO}_2$  content per liter of air.

Figure 2 gives a side view of the interior of the apparatus, showing the pots in which the plants are grown.

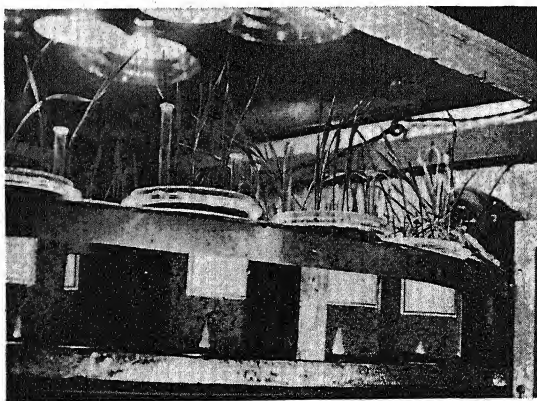


FIG. 2. Side view of the interior of the apparatus.

For technical reasons Gramineae have been selected as experimental plants. Wheat was used in most cases, as its comparatively broad leaves make it more suitable than the narrow-leaved grasses for this particular type of work. Rye, oats and barley have, however, also been shown to give satisfactory results. We have good reason to believe that the results obtained with these plants also hold good for meadow and pasture grasses.

The experimental plants were examined at four different stages of growth, *viz.*, after 13, 18, 25 and 30 days. The estimation of the absorption of carbon dioxide was carried out in the apparatus by GASSNER and GOEZE (8), cut leaves being used for this purpose. The method is based on the determination of the quantity of  $\text{CO}_2$  absorbed by the surface of the leaf.

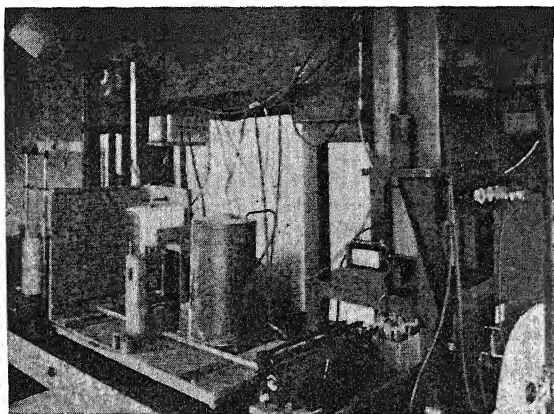


FIG. 3. The assembly for determining carbon dioxide assimilation.

The absorption of the carbon dioxide by the leaves takes place in the assimilation chamber. The latter consists of a narrow rectangular glass cell, which can be sealed by means of a metal cap. Through glass tubes fitted into the metal cap, air of known  $\text{CO}_2$  content is allowed to flow at a constant rate from a large gasometer into the assimilation chamber. When the air emerges it is led into a small gasometer of 3 liters capacity standing over glycerol. From the latter the air is filled into evacuated flasks of known capacity and its  $\text{CO}_2$  content estimated by the baryta method.

As the temperature must be kept constant during assimilation the cell containing the leaves is immersed in a water bath with glass walls, which is kept at  $20^\circ \text{C}$ . by a constant flow of cold water. The light necessary for the assimilation process is supplied by two 1000-watt lamps placed on either side of the assimilation chamber, so that both the upper and lower sides of the leaves receive the same amount of light.

Six leaves are used for each estimation and are fixed upright in a small glass boat with a mixture of lanolin and vaseline.

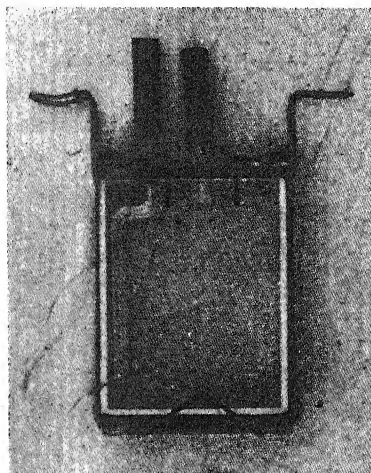


FIG. 4. Assimilation chamber closed (without assimilation frame).

The glass boat contains sufficient water to maintain the turgor in the leaves. The boat is weighed immediately before and after each estimation in order to determine the loss of water. The amount of  $\text{CO}_2$  absorbed by the leaves from 3 liters of air in five minutes is taken as a measure of the actual

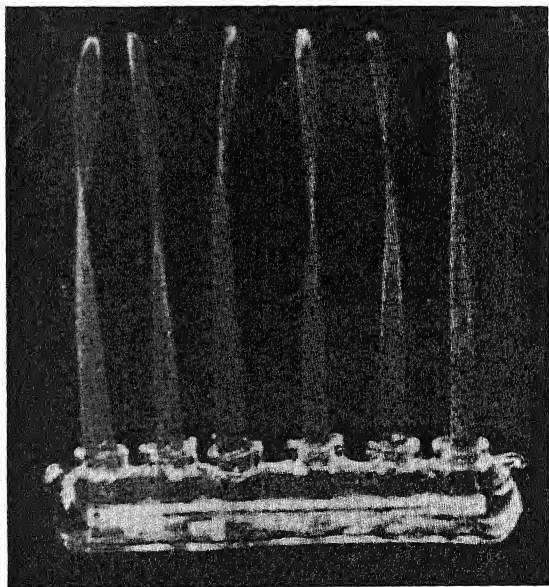


FIG. 5. Gramineae leaves in the glass boat during the estimation of assimilation and transpiration.

assimilation of  $\text{CO}_2$  by the plant. Before the actual estimation is carried out the leaves are exposed to the light from both lamps and air is allowed to flow through the assimilation chamber for 20 minutes in order that the leaves may become adapted to the experimental conditions. Three estimations are then carried out at intervals of five minutes on each set of leaves. The length and breadth of the leaves are then measured accurately. From each set of values for the area of the leaves, the time and the amount of  $\text{CO}_2$  absorbed, the quantity of  $\text{CO}_2$  in milligrams absorbed by 100 sq. cm. of leaf surface in one hour is calculated and the mean of the three estimations taken.

The chlorophyll content of the leaves is estimated in the acetone extracts using a Zeiss gradient photometer and a light filter no. S. 66.613.5. The experimental extracts are not compared with a standard chlorophyll solution as for the purposes of our investigations it is sufficient to determine the relative variation of the values. In cases in which absolute values for the chlorophyll content of the leaves are required, these can be obtained by comparison with a standard chlorophyll solution. The protein and soluble nitrogen are estimated by the micromethods recommended by KEYSSNER and TAUBÖCK (14). The protein nitrogen is precipitated with trichloroacetic acid and the nitrogen content of the precipitate and filtrate determined separately by the Kjeldahl method.

After preliminary tests we selected v. RÜMKER's "Square Head" spring wheat, as the most suitable variety for our purposes. The wheat plants were grown in glass jars of 1-liter capacity, the substratum being glass sand, to which 1.5 per cent. purified peat mull had been added in order to improve its water-holding capacity. During the growth of the plants the sand-peat mixture was kept at 40 per cent. of its maximum water-holding capacity. Each pot received a dressing consisting of 20 mg.  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , one drop of a 10 per cent. iron chloride solution and 25 mg.  $\text{CaCO}_3$ .

The experiments were conducted in two series, one with a low basal dressing consisting of 20 mg. N as  $\text{Ca}(\text{NO}_3)_2$  and 20 mg.  $\text{P}_2\text{O}_5$ , as mono-calcium phosphate per pot, and the other with a high basal dressing consisting of 100 mg. N and 100 mg.  $\text{P}_2\text{O}_5$  per pot. In both series the effect of potash applied as potassium sulphate at increasing rates from 0–100 and 0–250 mg.  $\text{K}_2\text{O}$  per pot was studied.

In order to improve aeration each pot was fitted with an unsymmetrical glass U-tube of 5-mm. diameter with 5 holes pierced in the bend.

## Results

Before discussing the results it may be mentioned that the figures given in the tables represent the mean of three experimental values.

In figure 6 and table I the values obtained with increasing applications of potash in conjunction with a low basal dressing are compared with those

obtained with varying amounts of potash in conjunction with a high basal dressing. The leaves used in both cases were from 25-day-old plants.

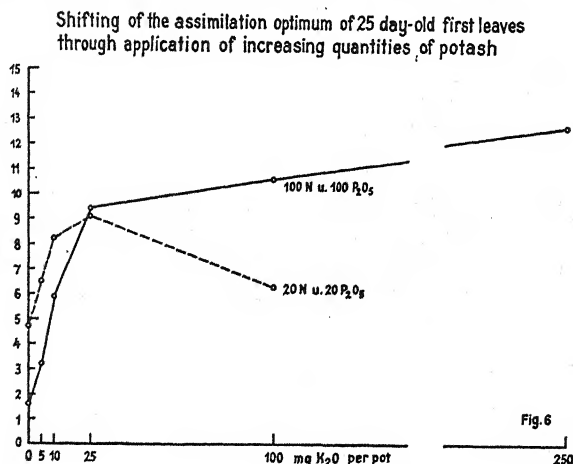


Fig. 6. The shifting of the assimilation optimum of 25-day-old first leaves through application of increasing quantities of potash.

TABLE I

CARBON DIOXIDE ASSIMILATION PER HOUR PER 100 SQ. CM. OF LEAF SURFACE

K <sub>2</sub> O PER POT	20 MG. N AND 20 MG. P <sub>2</sub> O <sub>5</sub>	100 MG. N AND 100 MG. P <sub>2</sub> O <sub>5</sub>
mg.	mg.	mg.
0 .....	4.7	1.6
5 .....	6.5	3.2
10 .....	8.2	5.9
25 .....	9.1	9.4
100 .....	6.3	10.6
250 .....	.....	12.7

As these figures show, the assimilation reached its maximum in the plants receiving 25 mg. per pot in conjunction with a low basal dressing, while in the series receiving a high basal dressing the maximum was not reached until the potash application was increased to 250 mg. K<sub>2</sub>O per pot; in the latter case the ratio N:K<sub>2</sub>O in the fertilizer was 1:2.5. In other words when heavy dressings of nitrogen and phosphoric acid are used, the potash application must be increased considerably in order to raise assimilation to its maximum.

The increase in the assimilation values with increasing applications of potash is not confined to one stage of growth only but may be observed in plants of all ages, as the following table shows.

TABLE II

ASSIMILATION BY WHEAT LEAVES OF DIFFERENT AGES (FIRST LEAVES) WITH INCREASING APPLICATION OF POTASH AND 100 MG. NITROGEN

K <sub>2</sub> O PER POT	13 DAYS	18 DAYS	25 DAYS	30 DAYS
mg.	mg.	mg.	mg.	mg.
0 .....	3.4	2.7	1.2	.....
5 .....	4.3	6.7	3.0	0.0
10 .....	6.5	11.1	6.6	3.1
25 .....	8.9	12.9	9.2	6.3
100 .....	(6.5)	13.9	11.4	6.8
250 .....	8.6	14.9	12.5	7.6

At all stages of growth at which the plants were examined, *viz.*, after 13, 18, 25, and 30 days, the increase in assimilation with increasing potash applications was rapid up to an application of 25 mg. K<sub>2</sub>O per pot, after which the rate of increase tended to slow down gradually.

The curves given in figure 7 show the relationship between assimilation and the age of the plants.

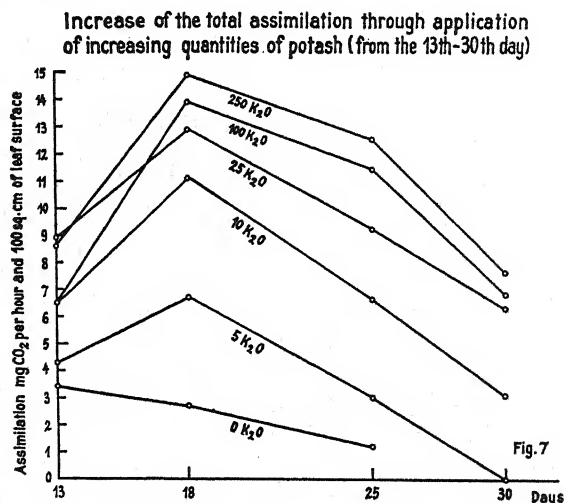


FIG. 7. Assimilation rates at different ages of the plants with respect to varying amounts of potash.

It is seen that apart from the "No Potash" series the assimilation values corresponding to the different potash treatments increase from the 13th day to the 18th day and then commence to fall; this falling off of assimilation with increasing age is however much more rapid in the case of the plants receiving the low potash dressings than in that of the "High Potash" plants. This is in accordance with the fact, often observed under practical condi-

tions, that the leaves of potash deficient plants cease to function normally much earlier than those of plants sufficiently supplied with this nutrient,—RICHARDS and TEMPLEMAN (20); SINGH and LAL (26); ECKSTEIN, BRUNO and TURRENTINE (5).

Figure 7 also shows the total assimilation of carbon dioxide by the leaves from each treatment during the growing period. This is represented by the area enclosed by the assimilation curve for the particular manuring. This area is greater the heavier the potash application to which the curve corresponds and reaches a maximum at 250 mg.  $K_2O$  per pot, which means that not only the intensity of assimilation but also that the actual production of vegetable matter must be greater in grasses receiving adequate amounts of potash than in potash deficient grasses.

The intensity of illumination during the growth of the experimental plants was not quite sufficient for optimal development, whereas, during the actual assimilation determinations, light conditions were optimal. For this reason one cannot say with certainty, that the differences in the assimilation of carbon dioxide by the differently treated plants are really so marked during the growth of the actual plants as the experimental values would lead one to believe. At all events it is remarkable that in spite of the inadequacy of illumination during growth, the leaves from the plants receiving liberal amounts of potash proved the most efficient in respect to assimilation when light conditions were improved. In this connection reference may be made to an observation of RUSSELL'S (21) that the response to liberal potash manuring is particularly marked in seasons lacking in sunshine. This effect of potash manuring has also been studied experimentally by SCHARRER and SCHROPP (22) and by FRANK (7). The ordinary type of experiment, in which accurate control of all growth factors is not possible, is hardly suited to such work. We hope, however, by means of the apparatus described above to contribute to the solution of this important problem.

The results of the experiments at present under discussion confirm and amplify observations made by other workers (18) that the efficiency of the assimilation mechanism of the plant is increased by increasing potash applications, independently of all other factors.

As has been stressed in the introduction to this paper, the different physiological processes are interdependent. On this account we supplemented our assimilation studies by investigations on the factors influencing transpiration, protein formation and chlorophyll content.

In order to facilitate the comparison of the effects of the different potash applications, the values obtained in each case have been expressed as percentages of those obtained from an application of 100 mg.  $K_2O$  in figure 8



and table III, in which the values represent those obtained with leaves from 25-day-old plants.

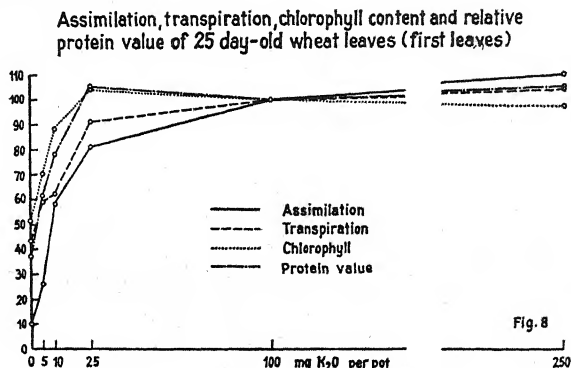


FIG. 8. Assimilation, transpiration, chlorophyll content and relative protein value of the first leaves of 25-day-old wheat plants.

TABLE III

QUARTZ SAND WITH HIGH BASAL MANURING (100 MG. N + 100 MG.  $P_2O_5$  PER POT)

$K_2O$ PER POT	ASSIMILATION $CO_2$ PER HOUR PER 100 SQ. CM. LEAF SURFACE	TRANSPIRATION $H_2O$ PER HOUR PER 100 SQ. CM. LEAF SURFACE	CHLOROPHYLL* CONTENT K PER 100 SQ. CM. LEAF SURFACE	RELATIVE PROTEIN VALUE†
<i>mg.</i>	<i>mg.</i>	<i>mg.</i>		
0 .....	1.2	355	1.01	1.7
5 .....	3.0	445	1.38	2.7
10 .....	6.6	465	1.74	3.5
25 .....	9.2	685	2.05	4.6
100 .....	11.4	750	1.97	4.4
250 .....	12.5	780	1.91	4.6

\* K = photometric extinction coefficient.

† Ratio of protein N to soluble N.

In our investigations of the effect of potash manuring on transpiration we found that transpiration increases with increasing applications of potash and reaches its maximum at 250 mg.  $K_2O$ . The transpiration curve is therefore similar in type to the assimilation curve, as is evident from figure 8.

The manner in which these two values are connected has not yet been definitely ascertained. STÅLFELT (30) found that when the diameters of the stomata lie between 2 and 4  $\mu$  both transpiration and assimilation are dependent on the diameter of the stomata. As a result of similar investigations GASSNER and GOEZE (9) arrived at the same conclusion. In connection with the assimilation studies the diameters of the stomata of wheat leaves were determined directly by means of the microscope after the method of STÅLFELT (29). The leaves used for this purpose were, as in the assimilation studies, exposed in the assimilation chamber to the light from

the two 1000-watt lamps for 20 minutes and then examined under the microscope. Measurements were taken of 10 stomata on the upper and lower surfaces of the leaves and it was found that, independent of manurial treatment, the diameters of the stomata on the upper surfaces of the leaves lay between 2 and 3  $\mu$  and of those on the under surfaces between 1-2  $\mu$ . This would indicate that the diameter of the stomata was not influenced by potash applications increasing from 0 - 250 mg.  $K_2O$ , although the values obtained correspond to the limits within which, according to STÅLFELT (30), both assimilation and transpiration show a rising tendency. Measurements carried out on leaves taken directly from the experimental plants yielded similar negative results. Working with mustard plants growing in water cultures MÜLLER and LARSEN (18) were also unable to establish any correlation between the diameter of the stomata and the changes in the production of organic matter in the plant due to potash and nitrogen deficiencies.

It is intended however to repeat these investigations on a greater number of plants with a view to verifying the foregoing results. Furthermore the mutual relationship existing between diameter of the stomata, water economy and the effect of potash is a question which has still to be investigated thoroughly. The results obtained in the investigations on the effect of potash on the water economy of plants, carried out up till the present, are rather conflicting. GASSNER and GOEZE (9) as well as SCHMALFUSS (25) emphasize the fact, that in most of these experiments the intake by and loss of water from the plant have not been studied separately, so that the observed loss in weight represents only the resultant effect of both processes. The contradictory nature of the results obtained may at least in part be attributed to the variety of methods used by these workers. On the other hand the particular combination of nutrient salts used is also of importance; for instance working with water cultures HANSTEEN-CRANNER (12) claims to have observed an increase, and SNOW (28) a decrease, of transpiration due to potash deficiency. The importance not only of the absolute amounts of  $K_2O$  and N used but also of the ratio in which they are supplied to the plant, has been already stressed sufficiently in the discussion of the influence of potash manuring on assimilation.

In our experiments transpiration was found to proceed parallel to assimilation. In plants receiving sufficient nitrogen both increase with increasing applications of potash, while in nitrogen deficient plants both tend to be more or less markedly depressed by heavy potash manuring.

These results obtained with cut leaves correspond to those obtained by SCHIECK (23), GASSNER and GOEZE (9) and ALTEN and GOEZE (3), which showed that any improvement in conditions with respect to nutrition produced a corresponding increase in transpiration; they do not however permit of any conclusions being drawn from them in regard to the influence of

nutrition on the water economy of the plant. It remains for further investigations to establish a relationship between the above results and the physiological functioning of the stomata on the one hand and the results of practical experience in respect to the effect of manuring on the consumption of water by agricultural crops on the other.

The same material, as used in the assimilation and transpiration studies, was also utilized for the protein studies. The results obtained are given in table III and figure 8.

In these investigations the ratio protein nitrogen/soluble nitrogen, the so-called "relative protein value," PAECH (19), was adopted as a criterion of protein formation. This value expresses the momentary state of equilibrium in protein metabolism and therefore the capability of the leaves to synthesize proteins. A low relative protein value indicates a high content of soluble nitrogen and in such cases the efficiency of the leaf for building up protein is impaired, so that the breaking down of the proteins tends to predominate. This can occur, for example, in older leaves as shown by MOTHES (16, 17) and SMIRNOW (27).

As may be seen from figure 8 the relative protein value of grasses increases with increasing potash applications. In contrast to the assimilation values the protein value reaches its maximum in plants receiving 25 mg.  $K_2O$  and then remains constant even with the highest dose of potash fertilizer. In grasses suffering from a marked deficiency of potash, the functional disturbances producing low assimilation values are evidently also accompanied by disturbances of the processes involved in protein formation.

The relationship between assimilation and protein metabolism will be discussed later in connection with their mutual dependence on the age of the plant. As the experimental values (figure 8) indicate the chlorophyll content at first increases with increasing applications of potash, similar to assimilation, but in contrast to the latter reaches its maximum at 25 mg.  $K_2O$  and falls slightly when the potash application is increased beyond this limit.

In agricultural practice special value is often attached to a high chlorophyll content of the leaf and attempts have been made to correlate chlorophyll content with the probable height of yield. That a relationship exists between chlorophyll content and assimilation has been demonstrated by EMERSON (6) and others and the results obtained by GASSNER and GOEZE (9) point in the same direction. The latter authors have pointed out, however, that assimilation may be very weak in the presence of a high chlorophyll content, while WILLSTÄTTER and STOLL (31) have produced evidence to show that a direct proportionality need not exist between chlorophyll content and assimilation. In this connection see also MÜLLER and LARSEN (18). This is also indicated by the experimental values given in figure 8,

which show that the leaves from plants receiving 250 mg.  $K_2O$  had a comparatively low chlorophyll content. From this it would seem that high potash applications tended to depress the chlorophyll content of the leaf. Similar observations have been made by MAIWALD (15) on potato plants. On the other hand our results also show that a depression of the chlorophyll content does not mean that the functioning of the leaf, in this case the synthesis of organic matter, is impaired.

Conversely it can also happen that grasses in poor condition may exhibit a high chlorophyll content. A very typical case is presented by the results obtained with the second leaves, as can be seen from figure 9 and table IV.

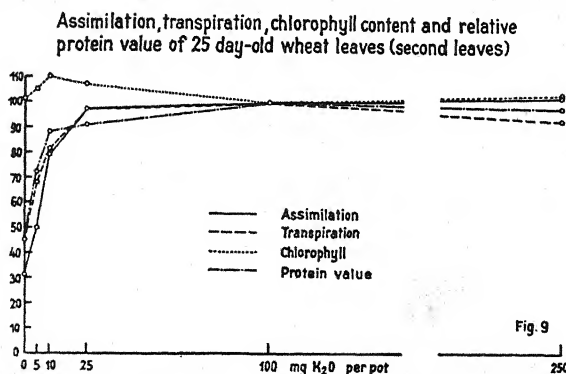


FIG 9. Assimilation, transpiration, chlorophyll content and relative protein value of the second leaves of 25-day-old wheat plants.

TABLE IV

ASSIMILATION, TRANSPIRATION, CHLOROPHYLL CONTENT AND RELATIVE PROTEIN VALUE OF THE SECOND LEAVES OF 25-DAY-OLD WHEAT PLANTS

$K_2O$ PER POT	ASSIMILATION OF $CO_2$ PER HOUR PER 100 SQ. CM. LEAF SURFACE	TRANSPIRATION $H_2O$ PER HOUR PER 100 SQ. CM. LEAF SURFACE	CHLOROPHYLL* CONTENT K PER 100 SQ. CM. LEAF SURFACE	RELATIVE PROTEIN VALUE†
mg.	mg.	mg.		
0 .....	3.7	260	2.16	1.9
5 .....	6.0	395	2.25	3.1
10 .....	9.6	470	2.36	3.8
25 .....	11.8	565	2.28	3.9
100 .....	12.1	580	2.14	4.3
250 .....	12.4	540	2.20	4.4

\* K = photometric extinction coefficient.

† Ratio of protein N to soluble N.

In this case the highest chlorophyll content corresponded to the lower potash applications, while the same leaves gave very low assimilation values. In spite of the high chlorophyll content, which imparted a dark green color to the leaves, the functioning of the latter was impaired. Neither a dark

accepted without reserve as being a reliable indication of a high production of organic matter.

The transpiration and relative protein values display the same characteristics as in the case of the first leaves, that is they increase parallel to the assimilation values with increasing applications of potash. In contrast to the chlorophyll values they also give evidence of functional disturbances in the case of plants receiving inadequate amounts of potash.

The extent to which assimilation and protein values are dependent on the age of the leaves will be dealt with briefly. Curves showing this relationship for plants receiving 250 mg.  $K_2O$  are given in figure 10, the different values for both assimilation and protein content being expressed as percentages of the maxima.

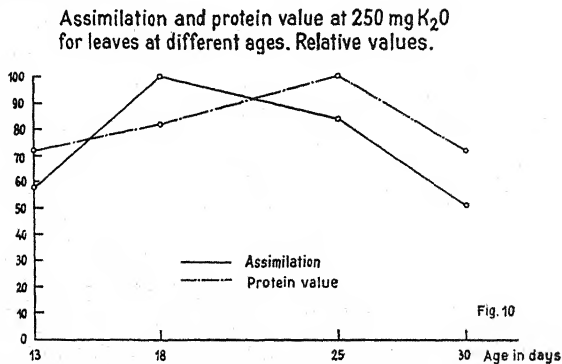


Fig. 10. Assimilation and protein values for leaves at different ages from plants supplied 250 mg.  $K_2O$ .

Whereas assimilation reaches its optimum in 18 days, the optimum for the relative protein values is reached after 25 days. Prior to this stage assimilation has already begun to fall off, *i.e.*, the functioning of the assimilation apparatus is impaired; nevertheless the protein value continues to rise until the 25th day and does not start to fall until the assimilation value has sunk to 85 per cent. of the optimum.

DESSAUERSTRASSE 28/29

BERLIN, GERMANY

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## ESTIMATION OF PROTOPLASMIC PERMEABILITY FROM PLASMOLYTIC TESTS

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Cell permeability as estimated by plasmolytic and other methods, while it may not go far to explain the problems of active absorption and translocation of materials, is none the less a highly significant factor in various physiological phenomena. Recent investigations by co-workers of the writer have shown, for example, that increase of cell permeability plays an important part in some quite unsuspected connections, *viz.*, in frost resistance (LEVITT and SCARTH, 1), drought resistance (WHITESIDE, unpublished results) and infection by rusts (THATCHER, 3). It would seem therefore that estimation of permeability might well be more commonly applied to the study of physiological problems, and the purpose of the present paper is to give some hints on technique and calculation which may encourage and aid this type of research. Its more particular purpose is to give formulae for the estimation of what COLLANDER calls protoplasmic permeability, *i.e.*, permeability per unit area of protoplast. Commonly the immediate research problem in hand requires only a knowledge of relative permeability, but as will be demonstrated, little extra labor is required to furnish an "absolute" value which is independent of the size, shape and osmotic pressure of the particular cells to which it refers. Accumulation of data in this form would gradually build up a body of knowledge of comparative cell physiology.

### Principles of permeability estimation

It may be necessary at the outset to defend the validity of plasmolytic methods. Under such treatment do cells behave as passive osmotic machines? Some tissues in fact do not. Shock effects appear in the form of "false plasmolysis" or "vacuolar contraction"—phenomena not governed apparently by simple osmotic relations. Unless recovery is rapid such tissues are unsuited to the technique—but they are the exception not the rule. More commonly we find occasional cells affected near the margin of a preparation which otherwise behaves normally. The abnormal cells may refuse to deplasmolyze with the others and often show a different color when stained with neutral red. Such cells have to be ignored. The fact remains, however, that as a rule cells do respond to osmotic changes in the environment in the way which is to be expected of them as sacs which are semipermeable to certain solutions and not to others.

Another question asked is: Do the results obtained by plasmolysis apply to cells under more normal conditions? With certain reservations, the writer believes that they do apply as regards "passive permeability." Thus,



differences in water permeability as displayed in plasmolysis tests are paralleled by the rate at which water passes out of cells on freezing. Solute permeability also is the same whether measured with or without plasmolysis. Also the osmotic value of the cell sap is usually found to be unchanged at the end of a permeability test. The reservations referred to are two mechanical effects which may temporarily produce abnormal permeability. One is too violent separation of the protoplast from the wall when there is a strong tendency to adhesion as shown by irregular plasmolysis shapes, and the other is too rapid deplasmolysis especially when the protoplast is highly viscous. Both effects can be avoided, the first by preliminary gradual plasmolysis and the second by slowing down deplasmolysis.

The far-reaching results of many prominent investigators are proof of the value of plasmolytic methods. No other technique has contributed so much to our knowledge of osmotic and permeability relations—witness the contributions of OVERTON, DE VRIES, FITTING, and the modern schools of HÖFLER and COLLANDER, to mention only a few. No other technique is so generally applicable or so speedy. Of course it must be learned and must always be used with judgment. It must be admitted, however, that quantitative estimates which take account of all the factors affecting the results are few indeed. An analysis of these factors is therefore our next step.

All plasmolytic methods determine permeability from the change over a known time in the volume of the protoplast. The volume change is either measured directly or estimated from osmotic change. Convenience usually decides that an increase rather than a decrease of volume is used. Hence the cells are first plasmolyzed and then allowed to deplasmolyze. The rate of volume change at any moment  $\frac{dv}{dv}$  is expressed by the following general formula

$$\frac{dv}{dt} = Pcs \dots\dots\dots (1),$$

where P = the coefficient of permeability of the protoplasm,

c = the difference of effective osmotic pressure or concentration between the inside and outside of the protoplast, and

s = the area of the surface of the protoplast.

Regarding the factor P we might expect *a priori* that it would be affected by change in the thickness of the protoplasmic layer during plasmolysis and deplasmolysis. It has been shown, however, that *water* permeability is unaffected by the degree of plasmolysis and, although in rapid deplasmolysis there is a temporary increase of permeability, this depends on the *rate* of expansion not the *stage* of deplasmolysis (LEVITT, SCARTH and GIBBS, 2). Thus while the value obtained by deplasmolysis may sometimes be too high it ought to remain constant as long as the rate of expansion is constant. In

tests for *solute* permeability deplasmolysis is very gradual and does not usually affect permeability. The constancy of  $P$  during deplasmolysis in urea solution has been proved for cells of leaf sheath of wheat by FRASER in this laboratory (unpublished results). It was found that with long narrow cells the length of the protoplast was a linear function of the time, which, as we shall see later, demands that  $P$  do not vary. We shall therefore regard  $P$  as remaining constant during the test, while  $c$  and  $s$  are variables.

The factor  $c$  in our equation should strictly be the *gradient* of concentration or pressure, but inasmuch as in the case of a cell the thickness of the effective membrane is unknown and is moreover apparently unaltered by change in thickness of the protoplasmic layer as a whole (since  $P$  is unaltered) it is customary to regard the driving force as simply a function of a *difference* of concentration or pressure inside and outside the protoplast. Its value when water alone is able to pass through the protoplasm is evidently the difference between the total internal and external osmotic pressures. The inside osmotic pressure, or concentration, decreases as the volume of the protoplast increases; the external pressure remains unchanged.

In the presence of a penetrating solution the osmotic relations are more complex but the net result is the same. To elucidate we shall designate the component pressures by symbols.

Let  $o$  = the partial pressure of sap solutes,

$c_1$  = the partial pressure of penetrating solute inside,

$C$  = the partial pressure of penetrating solute outside, and

$A$  = the partial pressure of non-penetrating solute outside.

No other components can exist, and  $A$  is often absent. When water is being drawn into the deplasmolyzing protoplast the total osmotic pressure inside must exceed the total pressure outside to bring this about. Let the excess pressure inside =  $P$ . Then

$$o + c_1 - p = C + A$$

or

$$o - A = C - c_1 + p$$

Now  $C - c_1$  is the pressure responsible for the entry of *solute* and  $p$ , by definition, that responsible for the entry of *water*. Therefore the sum of these is the total pressure causing the *solution* to enter, and this is seen to equal  $o - A$ , which is the same as when water alone can pass.

Thus  $c$  in equation (1) may be replaced by  $o - A$  whether  $P$  stands for water permeability or "solution permeability," if we may use such a term, hence

$$\frac{dv}{dt} = P (o - A) s$$

It is to be noted that this formula gives only the *volume* of solution taken up. Before the rate of entry of *solute* can be determined it is necessary to

know either the *concentration* in which the solution enters or the partial pressure of the penetrating solute in the sap. The formula, however, is a first step in the estimation of solute permeability and further steps will be dealt with later.

Variation of the factor  $s$  is one of the complications inherent in the plasmolytic method. Important though surface area is, most authors have neglected it in the past. One reason for this neglect has been their uncertainty as to how much of the surface of the protoplasts is freely available in sections of tissue. If there is any blanketing as regards diffusion by contiguous cells this effect ought to be at a maximum in the middle of a section and be manifest by a lag in deplasmolysis in this region as compared with the marginal cells. With thin sections of parenchymatous tissue no such effect appears, however (THATCHER, 3). In such preparations evidently the whole surface of every protoplast is more or less equally utilized in osmosis—at least until deplasmolysis is almost complete—and the permeability of its own protoplasm is so far the limiting factor for each cell that other interference is negligible. This condition cannot hold so completely in epidermal preparations, but for the great bulk of plant tissue its attainment is probably near enough, provided the section is for the most part only one cell thick.

The interrelationship of the factors  $v$ ,  $c$ , and  $s$  has now to be considered because equation (1) is insoluble unless  $c$  and  $s$  (which are variables) can be expressed in terms of  $v$ . The relation of  $c$  to  $v$  always remains constant. The value of  $c$ , as we have seen, equals  $o - A$ .  $A$  is constant and  $o$  varies

as  $\frac{1}{v - x}$  where  $x$  is the "non-solvent space." Any change in  $x$  which may be produced by change in volume is a quite negligible one in its bound-water fraction. Very commonly  $x$  itself can be neglected, but in cells rich in colloid, as in embryonic tissues and those adapted to withstand extreme drought or frost, it may be a large part of  $v$ .

The relation of  $s$  to  $v$  depends on the shape of the cell cavity combined with the ability of the protoplast when it leaves the wall to be molded by surface tension. As a rule a minimal area form is not assumed during the process of plasmolysis but is reached either on standing in the plasmolyte, or at least after a slight degree of subsequent deplasmolysis. This is one of the reasons for preferring the deplasmolysis phase of volume change for measurement of permeability.

As long as the protoplast retains the same geometric form,  $s$  can be expressed as a function of  $v$ . With isodiametric cells the spherical form is assumed and preserved up to a certain point, which is usually considerably short of complete deplasmolysis. For a sphere  $s = (4\pi)^{1/3} \cdot (3v)^{2/3}$ . The commonest shape of cell in plant tissues, however, has one axis longer than the others: it is cylindric or cylindro-prismatic and here the protoplast keeps its

form until the cavity is nearly filled. The form in question is approximately that of a cylinder with hemispherical ends in which

$$s = \frac{2v}{r} + \frac{4}{3} \pi r^2,$$

$r$  being half the diameter of the cell and therefore constant. For purpose of precise calculation it is necessary to regard the upper limit of volume in which either of the above geometric forms is preserved rather than the full volume of the cell, as the standard to which we refer as "incipient plasmolysis" or "end plasmolysis" as the case may be. With this limitation  $s$  may be expressed as a steady function of  $v$  during plasmolysis of the great majority of plant cells.

Having reached the conclusion that  $P$  does not change during a properly conducted deplasmolysis test and that the variables  $c$  and  $s$  can be kept as constant functions of  $v$  we are left with the relation of  $v$  to  $t$ , which must be learned from experiment. If only relative values of  $v$  and  $t$  are given in the equation, the constant  $P$  will stand for relative or "protoplast permeability"; if their absolute values are used,  $P$  represents "protoplasmic permeability," a much more definite property. Our next step therefore is to solve the basic equation and derive formulae which are convenient for quantitative experiments with the principal types of cell.

#### FORMULAE FOR CYLINDRIC CELLS

DERIVATION OF A GENERAL FORMULA.—Let us consider equation (1) as it applies to cylindrical cells of all kinds.

Let  $V$  = volume of the protoplast at "incipient plasmolysis,"

$O$  = osmotic value at volume  $V$ ,

$L$  = full length of the cell,

$l$  = length of the protoplast at volume  $v$ ,

$x$  = non-solvent space,

$y = v - x$ , and

$Y = V - x$ .

For the sake of simplicity we exclude here the presence of a non-penetrating solution outside, a condition dealt with later.

We have seen that  $s$  and  $c$  are constant functions of  $v$  and hence of  $y$  namely,

$$s = \frac{2(v + \frac{2}{3}\pi r^3)}{r} = \frac{2(y + x + \frac{2}{3}\pi r^3)}{r}$$

and

$$c = \frac{Y}{y} O; \text{ also } dy = dv.$$

Substituting in equation (1),

$$\frac{dy}{dt} = \frac{2POY}{r} \left( 1 + \frac{x + \frac{2}{3}\pi r^3}{y} \right)$$

$$= a + \frac{b}{y}, \text{ where } a = \frac{2POY}{r},$$

$$\text{and } b = \frac{2POY}{r} \left( x + \frac{2}{3}\pi r^3 \right).$$

Separating the variables and integrating,

$$\int \frac{y}{ay + b} dy = \int dt$$

$$\frac{1}{a^2} \left[ b + ay - b \log_e (b + ay) \right] = t + C.$$

To evaluate the constant of integration C, let  $v_0 = v$  when  $t = 0$  whence

$$C = \frac{b + ay_0}{a^2} - \frac{b}{a^2} \log_e (b + ay_0).$$

Substituting for C in the previous equation,

$$\frac{b}{a^2} \log_e \frac{b + ay_0}{b + ay} + \frac{b + ay}{a^2} = t + \frac{b + ay_0}{a^2}$$

Restoring the equivalents for a and b and substituting  $v - x$  for y this becomes

$$P = \frac{r}{2tO} \left( \frac{v - v_0}{V - x} - \frac{x + \frac{2}{3}\pi r^3}{V - x} \cdot \log_e \frac{\frac{2}{3}\pi r^3 + v}{\frac{2}{3}\pi r^3 + v_0} \right) \dots \dots \dots (2).$$

This equation may be further modified according to the data which the experimental technique provides. Two methods are in use, the "end plasmolysis" and the "plasmometric," though to estimate absolute permeability when non-solvent space is present the former method requires almost as much measurement as the latter.

With the "end plasmolysis" or "deplasmolysis time" method the initial and final volumes of the protoplast are fixed. The initial is the minimum volume which it assumes in the plasmolyte and when non-solvent space is negligible it is simply calculated from the known concentration of the plasmolyte. When, however, non-solvent space is appreciable the minimum volume must be measured and the technique becomes a special case of the plasmometric. The final volume is that at "incipient plasmolysis," which can be estimated at leisure from the length and diameter of the whole cell or average of cells in a section.

There are certain advantages in using the plasmolyte at an osmotic value which bears a definite ratio to that of the cell at incipient plasmolysis which we call O. This is better than always using the same concentration because it induces uniform changes in volume and surface area (as long as  $x$  is constant or negligible) so that if the complications of blanketing and expansion effect are not eliminated they tend to operate equally in all tests. Besides, the procedure simplifies calculation. If the value *twice* O be chosen and if the volume of the cell in equilibrium with the plasmolyte be  $v_{\min.}$ , then in equation (2)  $x = 2 v_{\min.} - V$ . In the following formulae the osmotic pressure of the plasmolyte will be taken as equal to  $2 \times O$ .

A formula for the deplasmolysis time method is obtained from equation (2) by putting  $V$  for  $v$ ,  $v_{\min.}$  for  $v_0$ , and  $2v_{\min.} - V$  for  $x$ . It is also more convenient to express  $v$  as  $(1 - \frac{1}{3}d)\pi r^2$ . This gives us

$$P = \frac{d}{8tO} \left[ 1 - \frac{2l_{\min.} - L}{L - l_{\min.}} \log_e \frac{L}{l_{\min.}} \right] \dots \dots \dots (3).$$

The plasmometric technique proper differs from the deplasmolysis time method merely in that *any* two or more lengths of the protoplast are measured at known time intervals. If these lengths be  $l_1$  and  $l_2$  we get the following formula for the plasmometric method:

$$P = \frac{d}{8tO} \left[ \frac{l_2 - l_1}{L - l_{\min.}} - \frac{2l_{\min.} - L}{L - l_{\min.}} \cdot \log_e \frac{l_2}{l_1} \right] \dots \dots \dots (4).$$

The plasmometric technique is better adapted to measuring solute than water permeability and is the only legitimate one for solutes which show an extremely slow rate of penetration.

**SIMPLIFICATION WHEN  $x$  IS NEGLIGIBLE.**—As non-solvent space is so commonly negligible in plant cells we shall consider particularly how equation (2) may be simplified for this case and for use with different experimental techniques.

*Deplasmolysis time method.*—When  $x=0$  and the cells are plasmolyzed in a solution of  $2 \times O$ ,  $v_0$  (i.e.,  $v_{\min.}$ ) =  $\frac{1}{2}V$ . Substituting these values and also  $V$  for  $v$  in equation (2) we get

$$P = \frac{d}{8tO} \left( 1 - \frac{2d}{3(L - \frac{1}{3}d)} \log_e \frac{2L}{L + \frac{1}{3}d} \right) \dots \dots \dots (5).$$

Values of the last term in the above equation for various  $\frac{L}{d}$  ratios are given in the following table.

TABLE I

$\frac{L}{d}$	$\left[ 1 - \frac{2d}{3(L - \frac{1}{3}d)} \log_e \frac{2L}{L + \frac{1}{3}d} \right]$	$\frac{3(L - \frac{1}{3}d)}{3L + \frac{1}{3}d}$
2 .....	0.78	0.79
3 .....	0.85	0.86
4 .....	0.89	0.89
5 .....	0.91	0.91
6 .....	0.92	0.92
7 .....	0.93	0.93
8 .....	0.94	0.94
9 .....	0.95	0.95

*Plasmometric method.*—For the plasmometric method using the same symbols as before we get,

$$P = \frac{d}{4tO} \left[ \frac{l_2 - l_1}{L - \frac{1}{3}d} - \frac{d}{3(L - \frac{1}{3}d)} \log_e \left( \frac{l_2}{l_1} \right) \right] \dots \dots \dots (6).$$

*Approximations.*—The experimental error is likely to be such that with cells having  $\frac{L}{d} \geq 4$  the following approximate formulae are adequate:

End-plasmolysis method,

$$P = \frac{d}{8tO} \dots\dots\dots (7).$$

Plasmometric method,

$$P = \frac{d(l_2 - l_1)}{4tOL} \dots\dots\dots (8).$$

*Relative permeability.*—For the same size and shape of cell the following simple formula is accurate for relative permeability as tested by end plasmolysis:

$$K = \frac{1}{tO},$$

where  $K$  is a constant factor for permeability.

FORMULAE FROM A SIMPLE EQUATION.—If the rate of volume change is constant there is no reason to use calculus because the following simple equation is equally valid:

$$P = \frac{v - v_0}{tc_m s_m} \dots\dots\dots (9),$$

where  $c_m$  and  $s_m$  are the effective pressure difference and the surface area respectively at the mean volume  $\frac{v + v_0}{2}$ . This condition is approached when

$\frac{L}{d}$  is high. To discover the error at lower  $\frac{L}{d}$  rates we compare the results with those obtained from equation (5) and table I. When  $x=0$  and the concentration of the plasmolyte is  $2 \times O$ , equation (9) applied to end plasmolysis becomes

$$P = \frac{3d(L - \frac{1}{3}d)}{8tO(3L + \frac{1}{3}d)} \dots\dots\dots (10).$$

Here the expression  $\frac{3(L - \frac{1}{3}d)}{3L + \frac{1}{3}d}$  takes the place of the bracketed expression in equation (5). Comparing the values of these two factors as given in table I we see that even for  $\frac{L}{d}$  ratios of 2 and 3 the difference is quite negligible. It is to be noted that this close agreement of the approximate formula with the exact one is obtained by taking  $c_m$  and  $s_m$  *not* as the mean pressure difference and mean surface area respectively but as the values of these two factors *at the mean volume*.

We conclude from this near agreement that where the calculus form of equation is cumbersome or insoluble the simple form may safely be employed. Some cases in point will now be dealt with.

*Approximation when  $\frac{L}{d}$  and  $x$  are both large.*—When  $x$  is large the final term in equation (2) cannot be neglected in spite of a high  $\frac{L}{d}$  ratio. Equation (9), however, applied to the end-plasmolysis method with a plasmolyte of concentration  $2 \times 0$  gives a usable formula:

$$P = \frac{3(L - l_{min.})d}{8tO(L + l_{min.})} \text{ approx.} \quad (11).$$

*Deplasmolysis in a hypotonic non-penetrating solution.*—The difficulty in estimating water permeability is the speed with which deplasmolysis takes place, which not only makes measurement of  $t$  difficult but also produces an abnormal degree of permeability. To some extent this is overcome by using a not too hypotonic solution instead of water as the deplasmolyte. As calculus treatment of this condition for cells in general is complicated we again resort to equation (9).

When the plasmolyte has a concentration  $2 \times 0$  and the deplasmolyte a concentration of  $\frac{1}{2}0$

$$c_m = 0(4/3 - \frac{1}{2}) = \frac{5}{6}0.$$

Therefore for cylindric cells in general we get from (9)

$$P = \frac{3(L - \frac{1}{3}d)d}{5tO(3L + \frac{1}{3}d)} \text{ approx.} \quad (12),$$

and for cells with a high  $\frac{L}{d}$  ratio:

$$P = \frac{d}{5tO} \text{ approx.} \quad (13).$$

This latter condition can be treated easily by calculus and gives the result:

$$P = \frac{d}{2tO} \cdot \log_e 3/2 = \frac{0.405d}{2tO}$$

which is practically the same as (13).

#### FORMULAE FOR SPHERICAL PROTOPLASTS

*FORMULA WHEN  $x$  IS NEGLIGIBLE.*—Substituting the respective functions of  $v$  for  $c$  and  $s$  in equation (1) we get

$$\frac{dv}{dt} = PO \frac{V}{v} (4\pi)^{1/3} (3v)^{2/3}$$

Separating the variables and integrating,

$$\int v^{1/3} dv = \int (POV(4\pi)^{1/3} 3^{2/3}) dt$$

whence,

$$\frac{3}{4}v^{4/3} = (POV(4\pi)^{1/3} (3)^{2/3})t + C.$$

Let  $v = v_0$  when  $t = 0$ , then  $C = \frac{3}{4}v_0^{4/3}$

Substituting for  $C$  and transposing,

$$P = \frac{\frac{3}{4}(v^{4/3} - v_0^{4/3})}{tOV(4\pi)^{1/3} 3^{2/3}} \quad (14).$$



*Plasmometric method.*—Let  $r$ ,  $r_0$  and  $R$  be the radii at  $v$ ,  $v_0$  and  $V$  respectively.

Then from (14),

$$P = \frac{r^4 - r_0^4}{4tOR^3} \dots\dots\dots (15).$$

*Deplasmolysis-time method.*—Here  $r = R$  and if the concentration of plasmolyte be  $2 \times O$ ,  $r^3 = \frac{1}{2}R^3$  and hence  $r_0^4 = \frac{1}{2}^{4/3}R^4$ . Thus equation (15) becomes:

$$P = \frac{0.15R}{tO} \dots\dots\dots (16).$$

The same result, to the second decimal place, is obtained from the simple equation (9).

FORMULA WHEN  $x$  IS APPRECIABLE.—Adapted to the end plasmolysis method and the use of a plasmolyte of concentration  $2 \times O$  equation (9) may be written:

$$P = \frac{V - v_{\min.}}{t \frac{4}{3}O (4\pi)^{1/3} \left[ \frac{3(V + v_{\min.})}{2} \right]^{2/3}} \\ = \frac{0.4(R - r_{\min.}^3)}{tO(R + r_{\min.}^3)^{2/3}} \dots\dots\dots (17).$$

#### SOLUTE PERMEABILITY

According to the above formulae permeability to *solutions* is estimated from increase of volume irrespective of the proportion in which water and solute enter the cell. However, the rate at which the concentration of penetrating solute increases inside the cell must be known before permeability to the *solute* can be calculated. Following the nomenclature used in the opening discussion in which the term  $p$  signifies the excess pressure inside the cell which causes the entry of water, and also assuming that the external medium consists of the penetrating solution alone, we have the following relationship at any moment of deplasmolysis:

$$p = o + c_1 - C.$$

*Slow penetration.*—With slowly penetrating solutions it is permissible to neglect  $p$ , because, if permeability to water is great compared with that to solute, a very low value of  $p$  relative to  $C - c_1$  will allow the entry of water to keep pace with that of solute. When  $p$  is negligible it follows that  $C - c_1 = o$  and also, since virtual osmotic equilibrium exists, the external solution must enter as such, that is, in concentration  $C$ . Hence the rate at which the solute enters is given by the product of  $C$  and  $\frac{dv}{dt}$ , and if  $P_s$  be used to designate solute permeability

$$C \frac{dv}{dt} = P_s os,$$

and

$$P_s = CP.$$

Thus any of the above formulae for solution permeability gives solute permeability when multiplied by the concentration of the penetrating solution outside.

For example from equation (5) we get the following for use with the end-plasmolysis method when  $C = 2 \times O$ :

$$P_s = \frac{d}{4t} \dots\dots\dots (18).$$

with corrections according to table I if necessary alternatively from equation (10).

$$P_s = \frac{3d(L - \frac{1}{3}d)}{4t(3L + \frac{1}{3}d)} \dots\dots\dots (19).$$

For the plasmometric method we get from equation (6),

$$P_s = \frac{C(l_2 - l_1)d}{4tO(L - \frac{1}{3}d)} \dots\dots\dots (20).$$

If  $C = 2 \times O$

$$P_s = \frac{d(l_2 - l_1)}{2t(L - \frac{1}{3}d)} \dots\dots\dots (21).$$

With slowly penetrating solutes the plasmometric method obviates the need of estimating  $O$  and even of knowing the value of  $C$ . If the amount of solute that penetrates before the protoplast reaches minimum volume in the penetrating solution is negligible and if  $x$  also is negligible,

$$\frac{C}{O} = \frac{V}{v_{min.}} = \frac{L - \frac{1}{3}d}{l_{min.} - \frac{1}{3}d}$$

Substituting in equation (20) we get

$$P_s = \frac{d(l_2 - l_1)}{4t(l_{min.} - \frac{1}{3}d)} \dots\dots\dots (22).$$

*Rapid penetration.*—With more rapidly penetrating solutions a second approximation which takes count of  $p$  is suggested though its use has not yet been tried out.

The following simple equation is applicable to either the end plasmolysis or plasmometric method:

$$P_s = \frac{v_e c_{te}}{t(C - c_{im})S_m}$$

where the sub-index  $e$  indicates end values after time  $t$  and sub-index  $m$  mean values. As  $c_i$  cannot be determined directly by plasmolytic methods we substitute  $C - o + p$  with a view of determining  $p$ . When applied to end plasmolysis in a solution of concentration  $2 \times O$  the above equation then becomes

$$P_s = \frac{V(O + p_e)}{t(O_m - p_m)S_m} \dots\dots\dots (23).$$

where  $o_m$  is the value of  $o$  at the mean volume, namely  $\frac{4}{3} O$ ,  $p_m$  is the mean value of  $p$  during deplasmolysis, and  $p_e$  its value at the end.

Since  $p$  is the net pressure causing water to enter, its mean value is obtained roughly from the relation of the time taken for an equal extent of deplasmolysis in the solution in question ( $t_{sol}$ ) and in water ( $t_w$ ), respectively. For if we neglect the fraction of the volume increase occupied by solute,

$$p_m = o_m \cdot \frac{t_w}{t_{sol}}$$

Theoretically it would seem that  $p$ , after a brief and speedy rise at the beginning of deplasmolysis, must then fall off gradually as the solute gradient  $C - c_1$  declines, because its value depends on the rate that solute enters. But if  $p$  varies as  $C - c_1$  it varies as  $o - p$  and therefore as  $o$ . As an approximation therefore we calculate  $p_e$  from the formula  $p_e = p_m \frac{O}{o_m}$ . Also, since  $t_{sol}$  here equals  $t$ ,  $p_m = \frac{t_w}{t} o_m$ . Substituting in (23) we get

$$P_s = \frac{3 \left(1 + \frac{t_w}{t}\right) V}{4t \left(1 - \frac{t_w}{t}\right) s_m} \dots\dots\dots (24).$$

In the case of cylindrical cells with a high  $\frac{L}{d}$  ratio this becomes

$$P_s = \frac{d \left(1 + \frac{t_w}{t}\right)}{4t \left(1 - \frac{t_w}{t}\right)} \dots\dots\dots (25).$$

For any solution the value of the fraction  $\frac{t_w}{t}$  decides whether a formula, such as the above, or the simpler one for slowly penetrating solutions should be used.

#### UNITS OF PERMEABILITY VALUE

The result for water permeability or "solution permeability" represents the rate of movement of liquid through the protoplasm per unit difference of concentration or pressure. A convenient mode of expression is microns per hour per atmosphere (since  $\mu^3/\mu^2 = \mu$ ).

As regards solute permeability, if concentration be expressed in mols, and cell dimensions in centimeters, the amount of solute taken up per  $\text{cm}^3$  will be given in *millimols*, since mols per litre (1,000 ml.) is equivalent to millimols per  $\text{cm}^3$ . The unit of concentration difference, however, is still the mol.

### Technique

A brief description of the experimental techniques which suit the preceding modes of calculation may be useful. If sectioning is required the sections must be longitudinal and generally about one cell thick.

A stock solution of  $\text{CaCl}_2$ , or of a "balanced" mixture of  $\text{NaCl}$  and  $\text{CaCl}_2$  (e.g. 9:1), is the best source for making up non-penetrating solutions. Salt penetrates cell walls more quickly than sugar and the salt solution keeps indefinitely in a well-stoppered bottle. This will be referred to as "standard salt solution." All experimental solutions should be preserved strictly from evaporation in covered dishes and the preparation mounted for observation only as long as is necessary.

### DEPLASMOLYSIS TIME METHOD

**STAINING.**—To facilitate observation, colorless cells are vitally stained in weak neutral red (5 to 50 parts per million). Slight alkalinity of the staining medium hastens penetration of the dye, and  $\text{Ca}$  ions prevent the cell wall from staining. Tap water often supplies both requirements. Staining is usually slow and incidentally allows time for recovery from the shock of cutting.

**OSMOTIC PRESSURE DETERMINATION.**—The average osmotic value ( $O$ ) of the cells at "incipient plasmolysis" (see previous discussion) is carefully determined from sections placed in a graded series of dilutions of the standard salt solution. The preparations, if they are to be used later for permeability tests, should not be left longer than necessary in the solution.

Unless non-solvent space is known to be absent, its operation must be estimated at this stage by determining the volume ( $V$ ) of the protoplast at incipient plasmolysis and then plasmolyzing in  $2 \times O$  salt solution and again

estimating the volume ( $v_o$ ). If  $\frac{V}{v_o} = 2$  we proceed as in the following paragraph; if less than 2, we proceed as described later.

**PERMEABILITY DETERMINATION.**—This method allows an average of all the cells in a section to be estimated to observe which natural or artificial coloration of the cells is almost essential.

**Water permeability.**—Sections are placed in a standard salt solution of concentration equal to twice  $O$  until plasmolysis is complete, say 10–15 minutes.

They are then transferred to a hypotonic solution ( $\frac{1}{2}O$ ) and the time of immersion noted. As soon after as experience indicates to be necessary, samples are mounted for brief intervals in a drop of the same solution for microscopic observation, and the moment at which deplasmolysis is complete in 50 per cent. of the cells is judged. This is rather difficult to estimate

sometimes because of the speed of the process and the wide individual variation among the cells. As already remarked, rapid deplasmolysis commonly causes a noticeable increase of permeability. Objections also attend the use of plasmolysis-time; but after the protoplasts have rounded up, it may at least be used as a check on the effect of deplasmolysis. Large cells and non-viscous protoplasm display least abnormality both in plasmolysis and deplasmolysis. The formula for elongate cylindrical cells is  $\frac{d}{5tO}$ .

For short cells correct from table I or use equation (12).

*Solute permeability.*—If the form of plasmolysis is irregular, with adhesion to the wall, the cells are first plasmolyzed in standard salt solution stronger than  $2 \times O$  and returned to  $2 \times O$  to give the protoplasts the required shape. Sections are then transferred to the penetrating solution having an osmotic pressure approximately equal to that of the  $2 \times O$  salt solution. Tables of osmotic pressure and concentration are invaluable in this connection. If, however, plasmolysis is smooth and produces convex contours the sections may be placed directly in the penetrant solution at the concentration  $2 \times O$ . The time elapsing from the moment of immersion in the penetrant to completion of deplasmolysis by about half of the cells is much easier to determine with slowly penetrating solutions than with water. The formula for elongate cells is  $\frac{d}{4t}$ . For short cells correct from table I or use equation (9).

#### PLASMOMETRIC METHOD

As this method can be applied accurately only to individual cells which have to be recognizable when the sections are remounted for later measurement, it is usually possible to select suitably long and narrow cells, which reduces the error of measurement and also simplifies calculation. Pre-staining is not essential with this method. If desired, the osmotic pressure of the selected cells is determined plasmometrically, that is, by measuring the length of the protoplast in equilibrium with a known concentration either of salt solution or even of the penetrant solution itself when the rate of entry of the solute is relatively slow; but it is not necessary to know the osmotic value, either of the cell or of the penetrant, if the minimum length is measured.

To estimate permeability plasmometrically, two or more lengths (or diameters if the form is spherical) of the deplasmolyzing protoplast are carefully measured with a micrometer at known time intervals. The minimum length may be one of these. The simplest formula for cylindrical cells is (22). This method allows greater accuracy than other plasmolytic methods but only as regards the cells actually measured.

NON-SOLVENT SPACE APPRECIABLE.—After the length  $l_{\min}$ . (or diameter) in  $2 \times 0$  salt solution has been measured, the cells are deplasmolyzed in whichever medium is suitable to the object in view. If solute permeability is to be determined, it is important that the osmotic pressure of the penetrating solution be as nearly as possible the same as that of the ( $2 \times 0$ ) solution. Disparity will be revealed by a quick change in protoplast volume when transferred to the penetrant solution.

### Summary

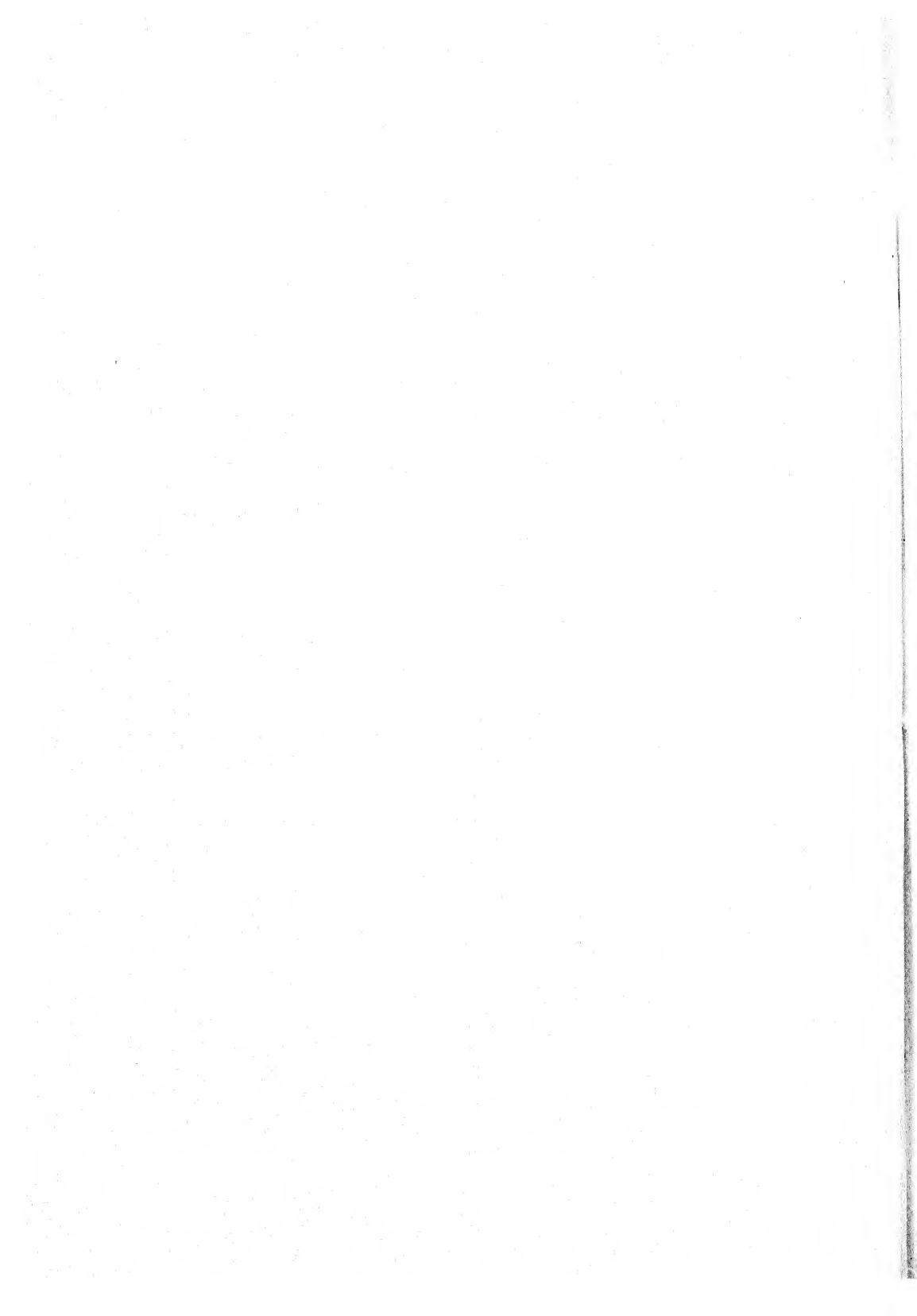
This paper includes a discussion of the principles of estimating protoplasmic permeability plasmolytically, the derivation of comprehensive formulae for use in estimating the protoplasmic permeability of cylindrical and spherical cells, and some notes on technique.

The writer is much indebted to Dr. W. ROWLES for aid with the mathematics and for useful suggestions.

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# EFFECT OF ETHYLENE ON CERTAIN CHEMICAL CHANGES ASSOCIATED WITH THE RIPENING OF PEARS<sup>1</sup>

ELMER HANSEN

(WITH TWO FIGURES)

## Introduction

Recent discoveries (7, 8, 10) have shown that ethylene occurs in small amounts as a natural metabolic product of certain fruit and other plant tissues. The fact that a gas of this nature is produced by plants is of great interest, especially in view of the fact that ethylene has been used extensively in the past as an artificial treatment to hasten ripening in various fruits. The results obtained from use of this treatment, however, have not always been uniform, and for this reason there apparently has developed a diversity of opinions as to the exact relation, if any, of ethylene to the ripening of fruit. In some cases respiration (6), starch hydrolysis (14), and other chemical changes are reported to have been affected. In other cases (5, 16, 26), the results obtained have indicated that there is no apparent benefit from the use of ethylene for increasing the rate of ripening in various fruits.

As an explanation for these differences, HARVEY (15) has suggested that the concentration of gas used in some experiments might have been too low. In other cases (1, 27) maturity of the fruit has evidently been a factor. It is apparent, however, that in many of these previous experiments the fact has been overlooked that during maturation and after storage, fruits naturally undergo progressive physiological changes which may greatly modify the response shown to ethylene treatment. Thus, in a former experiment with pears (12) it was found that the respiration of the fruit showed a definite, well-defined trend during maturation and after storage, and the response obtained with ethylene varied greatly according to the respiratory activity of the fruit at time of treatment.

In the present investigation it was considered desirable to study the effects of ethylene on certain chemical changes occurring in pears during the ripening process. By observing the comparative responses of a single variety treated at different stages of maturity and after various periods in cold storage, it was thought that any chemical changes which were definitely affected by this gas during any specific stage in the life of the fruit would become apparent. Information of this nature would be of value in determining what the effects of a natural accumulation of ethylene in fruit tissues would have upon initiation of the ripening process.

<sup>1</sup> Published as Technical Paper no. 285 with the approval of the Director of the Oregon Agricultural Experiment Station.



## Materials and general methods

### COLLECTION AND HANDLING OF SAMPLES

The fruit used in these experiments were Bartlett and Anjou pears, obtained from the Hood River and Medford (Oregon) districts. Fruit intended for treatment immediately after picking was gathered at approximately two-week intervals, beginning 5 weeks after blooming and extended several weeks beyond the regular commercial harvest season. Fruit intended for storage studies was picked on one date when the proper picking stage for the variety had been reached.

As soon as the fruit had been brought from the field or removed from storage, two uniform lots were sorted out and placed in 5-gallon glass jars. To one set of jars was added ethylene to make a concentration of 1:1000 by volume; the second set of jars was kept under constant ventilation with fresh air to prevent any accumulation of gases affecting ripening. Details of the method used to maintain comparable temperatures, humidities, and carbon dioxide and oxygen tensions in the treated and untreated jars are described in a previous publication (12). To compare the trend of chemical changes occurring after picking in the ethylene-treated and untreated lots, a sample of 15 pears was withdrawn for analysis at definite intervals during the course of ripening. With immature fruit, a sample was taken every 4 days during a period of 12 days. With mature and storage fruit, which ripened more rapidly, only two analyses were made: the first immediately after picking or removal from storage, and the second after a period of 8 days.

### Methods of analysis

**SAMPLING.**—Samples for chemical analysis were prepared by cutting longitudinal sectors of 15 whole pears, grinding them through a food chopper, and finally weighing out duplicate 50-gm. samples of the finely ground tissue.

**EXTRACTION.**—Sugars were extracted with 95 per cent. alcohol in a Soxhlet extractor for 24 to 30 hours. The alcohol was then distilled off under a 26 to 28 inch vacuum at 40° C., the water extract cleared with neutral lead acetate, de-leaded with potassium oxalate, and made to volume.

**SUGAR DETERMINATIONS.**—Reducing sugars before and after inversion were determined by the method of LANE and EYNON (19). Inversion was carried out by the method described by MARTIN (21) and sucrose was calculated by the usual method.

**ALCOHOL INSOLUBLE RESIDUE.**—The alcohol insoluble residue was determined as the dry weight of the material remaining in the thimbles after extraction.

**STARCH.**—This was determined on 0.5-gm. samples of the alcohol insoluble residue, finely ground with quartz sand, and digested with fresh saliva

according to the method described by LOOMIS and SHULL (20). The reducing values of the solutions after hydrolysis were determined by the iodometric method of SHAFFER and HARTMANN (24); and converted to starch by the factor 0.90.

**PECTIC SUBSTANCES.**—Soluble pectin and protopectin were determined by the method of CARRÉ and HAYNES (4) on 50-gm. samples of the fresh tissue, and are expressed as impure calcium pectate.

**ACID.**—Acid was determined on 50-ml. aliquots of the water solution of the alcohol extract used for sugar determinations and calculated as citric. Because of the difficulty in determining the end point with phenolphthalein indicator, the solutions were titrated electrometrically to pH 7.2 with 0.01N sodium hydroxide on a YODEN quinhydrone electrode.

## Results

### EXPERIMENTS WITH BARTLETT PEARS RIPENED BEFORE STORAGE

In order to have fruit representative of different stages of maturity, approximately 200 pears were picked at intervals throughout the later part of the growing season. The dates on which the samples were collected and the average weights of 25 fruits at time of picking are shown in table I.

TABLE I  
AVERAGE WEIGHT OF FRUIT COLLECTED AT DIFFERENT PERIODS

DATE PICKED	AVERAGE WEIGHT PER FRUIT
	<i>gm.</i>
July 14 .....	43.1
July 29 .....	99.3
August 16 .....	138.6
August 25* .....	150.5
September 14 .....	160.5

\* Commercial picking date.

The first picking, made on July 14, was extremely immature. The amount of ethylene produced at this stage of development was barely detectable by epinasty of potato leaves, and the tissues of the untreated fruit probably contained only traces of this gas naturally produced. Fruit picked later showed increasing evidence of ethylene production, and the tissues of the untreated fruit were probably not free from this gas, even though constant aeration was provided during the entire ripening period.

The changes in total and reducing sugars, sucrose, starch, pectic substances, alcohol insoluble residue, and acid are shown in table II, and are represented graphically, with the omission of acid and alcohol-insoluble residue, in figure 1. The percentages of increase or decrease in each material

TABLE II

EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARLETT PEARS PICKED AT DIFFERENT STAGES OF MATURITY

No. DAYS RIPENED	TOTAL SUGARS		REDUCING SUGARS		SUCROSE		SOLUBLE PECTIN		INSOLUBLE PROTOPECTIN		STARCH		ALCOHOL- INSOLUBLE RESIDUE		ACID AS CITRIC	
	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED
1st picking—July 14																
0 .....	4.20	4.20	4.06	4.06	0.13	0.13	0.10	0.10	0.85	0.85	1.24	1.24	6.65	6.65	0.23	0.23
4 .....	4.86	5.85	4.62	5.32	0.23	0.50	0.10	0.31	0.84	0.36	1.29	0.76	6.32	5.41	0.17	0.17
8 .....	5.29	6.39	5.18	5.80	0.11	0.56	0.12	0.65	0.80	0.25	1.14	0.21	5.98	5.91	0.28	0.27
12 .....	5.39	5.72	5.18	5.26	0.20	0.44	0.09	0.21	0.85	0.18	1.01	0.14	6.13	5.42	0.19	0.17
2nd picking—July 29																
0 .....	6.28	6.28	5.70	5.70	0.55	0.55	0.09	0.09	0.86	0.86	1.09	1.09	4.95	4.95	0.15	0.15
4 .....	6.54	6.82	6.08	6.43	0.44	0.38	0.08	0.57	0.90	0.42	0.85	0.60	4.70	4.45	0.18	0.19
8 .....	6.82	7.31	6.30	6.60	0.50	0.67	0.32	0.71	0.46	0.20	0.66	0.45	4.38	3.98	0.20	0.19
12 .....	7.72	7.60	7.15	7.00	0.55	0.57	0.51	0.38	0.13	0.10	0.39	0.28	3.54	3.59	0.23	0.23
3rd picking—August 16																
0 .....	6.68	6.68	6.04	6.04	0.61	0.61	0.21	0.21	0.64	0.64	0.71	0.71	4.35	4.35	0.20	0.20
4 .....	7.29	7.59	6.54	6.75	0.71	0.80	0.26	0.39	0.68	0.52	0.85	0.68	4.05	3.76	0.20	0.21
8 .....	7.72	7.58	6.99	6.52	0.70	1.00	0.42	0.64	0.26	0.10	0.60	0.34	3.63	3.39	0.22	0.19
4th picking—August 25																
0 .....	7.21	7.21	6.24	6.24	0.93	0.93	0.19	0.19	0.61	0.61	0.64	0.64	3.22	3.22	0.17	0.17
8 .....	8.42	8.44	6.67	6.90	1.66	1.46	0.51	0.59	0.23	0.19	0.37	0.10	3.22	3.38	0.22	0.21
5th picking—September 14																
0 .....	8.49	8.49	6.45	6.45	1.94	1.94	0.19	0.19	0.63	0.63	0.34	0.34	2.90	2.90	0.26	0.26
8 .....	8.87	8.97	6.49	6.56	2.26	2.29	0.65	0.68	0.19	0.15	0.21	0.19	2.42	2.41	0.15	0.21

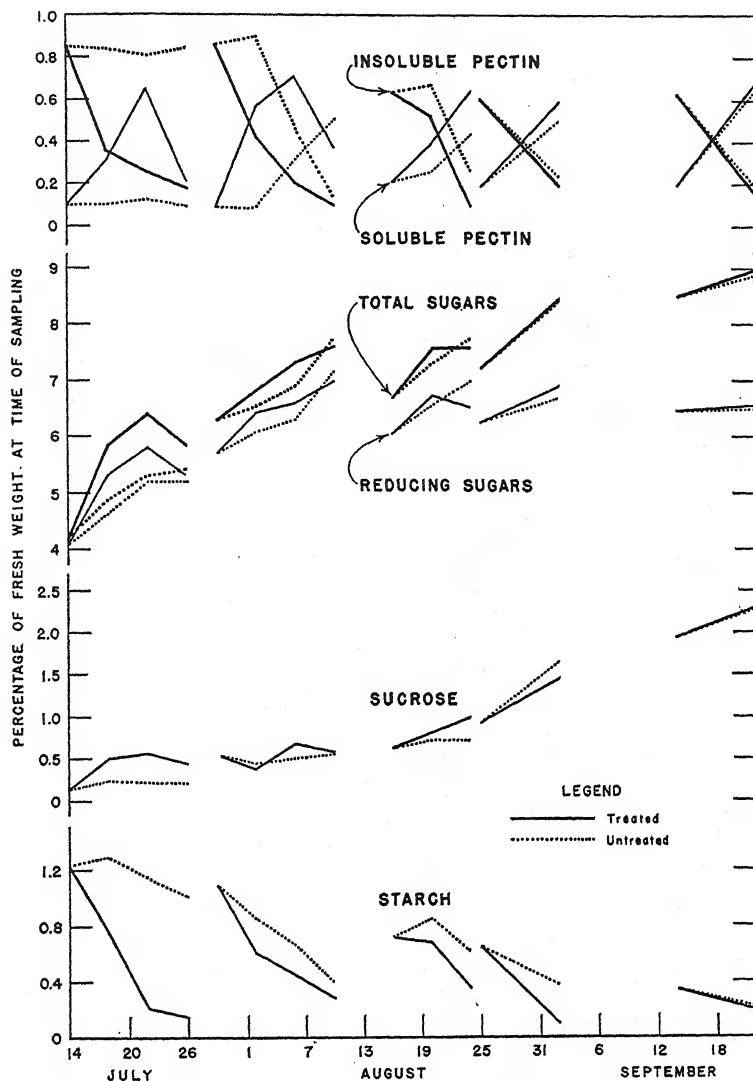


FIG. 1. Effect of ethylene on chemical changes of Bartlett pears picked at different stages of maturity.

during ripening of treated and untreated lots are shown in table III. These values were calculated on the basis of the percentage increase or decrease, at the end of 8 days, over or under the original amount of the material present at time of picking.

**CHANGES IN SUGARS.**—Total sugars in the green fruit increased throughout the growing season. During ripening, definitely greater increases were

TABLE III

COMPARATIVE CHANGES IN THE CONSTITUENTS OF ETHYLENE-TREATED AND UNTREATED BARLETT PEARS, EXPRESSED AS THE PERCENTAGE INCREASE OR DECREASE DURING EIGHT DAYS' RIPENING, OF THE AMOUNT ORIGINALLY PRESENT

INCREASE IN TOTAL SUGARS				INCREASE OR DECREASE IN SUCROSE			INCREASE IN SOLUBLE PECTIN			DECREASE IN INSOLUBLE PROTOPECTIN			DECREASE IN STARCH		
TREATED	UNTREATED	DIFFERENCE		TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE	TREATED	UNTREATED	DIFFERENCE
%	%	%		%	%	%	%	%	%	%	%	%	%	%	%
52.15	25.95	26.20		331.00	- 15.39	346.4	550.0	20.0	530.0	70.60	5.88	64.72	83.06	8.07	74.99
16.40	8.60	7.80		21.82	-	30.91	689.0	255.6	433.4	76.74	46.51	30.23	58.73	39.46	19.27
13.47	15.57	- 2.10		63.95	14.75	49.20	204.4	100.0	104.4	84.56	59.37	25.19	52.11	15.49	36.62
17.07	16.78	0.29		56.99	78.49	- 21.50	210.6	168.4	42.2	68.85	62.30	6.55	84.39	42.19	42.20
5.65	4.47	1.18		18.04	16.49	1.55	257.9	247.4	10.5	76.19	69.84	6.35	44.12	38.28	5.89

observed in the ethylene-treated than in the untreated lots in the first and second pickings; but in more mature fruit these differences were much less pronounced. As can be noted in table III, the increase in total sugars during ripening was 26 per cent. greater in the treated fruit of the first picking but only 1.18 per cent. greater in the last picking.

Reducing sugars showed changes similar to those found for total sugars. In the fruit of the first picking the increase in the treated sample was 15.27 per cent. over the untreated. In the final picking, however, an increase of only 1.09 per cent. was found in favor of ethylene treatment.

Very little sucrose occurs in immature green fruit but this sugar builds up constantly during maturation. During ripening more sucrose developed in the treated than in the untreated pears in all but one of the samples collected. In the first picking this increase amounted to over 300 per cent. in favor of ethylene treatment. Much smaller differences were found in fruit picked and ripened later in the season.

**STARCH.**—The maximum concentration of starch was found in the first sample collected, and the amount contained in the tissues at time of picking decreased thereafter. After ripening of the earliest collected samples, it became evident that ethylene treatment was very effective in increasing the rate of starch hydrolysis. In the treated samples of the first picking practically all of the starch had disappeared after a period of 8 days, while the untreated lot still retained all but a small fraction of the amount originally present. When the last fruit was collected late in the season, there was little starch remaining, and during ripening this disappeared almost as rapidly in the untreated as in the treated fruit.

**ALCOHOL INSOLUBLE RESIDUE.**—The amount of this material decreased throughout the growing season, and fruit treated early in the season contained less after ripening than did untreated fruit held for a similar period of time. These differences were not noticeable after ripening of more mature fruit picked at later dates.

**PECTIC CHANGES.**—The amount of soluble pectin in the green fruit at the time of picking was very small, never exceeding 0.2 per cent., even in pears collected two weeks later than the period when commercially mature. After picking and during ripening, the increase in soluble pectin closely parallels the increase in softening of the fruit and is, therefore, a very good index of progress in ripening.

That ethylene has a very pronounced effect on pectic changes is evident from the results obtained. Untreated fruit of the first picking was held for 12 days without any increase in soluble pectin. In the ethylene treated lot, however, there was a very rapid increase which at the end of 8 days amounted to more than 500 per cent. over the amount originally present.

In the untreated sample of the second picking, soluble pectin showed no increase during a 4-day period, but increased rapidly thereafter. In all fruit

collected at later dates, soluble pectin began to increase immediately after picking in the untreated lots, but the rate of this increase was always less rapid than that observed in the treated fruit. Even in the post-mature pears, the amount of soluble pectin developed was higher in the treated fruit, but the difference was much less than that found in earlier picked samples.

The amount of insoluble protopectin in the green fruit decreased slightly throughout the season. During ripening there was a further decrease which occurred more rapidly in the treated fruit. Judging from the data obtained, it is assumed that this decrease in insoluble protopectin is related to the increase in soluble pectin since the percentage decrease in one can approximately be accounted for on analysis as the percentage increase in the other.

The relation of protopectin to pectin has been investigated extensively in apples by CARRÉ (3). APPLEMAN and CONRAD (2) have reported that in peaches the rate of softening parallels the transformation of protopectin into pectin. Similar observations have been made on pears by EMMETT (9).

That ethylene increases the rate of softening in fruit is a common observation. As far as the writer is aware, however, specific data to show the effect of ethylene in increasing the rate of transformation of protopectin to pectin have not been reported previously.

ACID.—The amount of acid found in the Bartlett pears used in these experiments was small, never exceeding 0.3 per cent. During the course of ripening, the acid content showed considerable variation; and no well-defined trends in either treated or untreated lots were apparent. In some lots of fruit titratable acidity appeared to increase during ripening, but in most cases the changes observed were very small.

#### EXPERIMENTS WITH BARTLETT PEARS RIPENED AFTER STORAGE

The pears used for this series were held at a temperature of 31° F. Samples for analysis were withdrawn after periods of 10, 20, 30, 70, and 120 days, and then ripened at 65° F., as in all previous experiments. The chemical changes occurring in treated and untreated lots during ripening are shown in table IV.

CHANGES IN SUGARS.—Total and reducing sugars increased throughout the storage period. During ripening they showed a tendency to increase, but with the probable exception of the earliest withdrawn samples there were no significant differences observed between treated and untreated lots.

Sucrose increased during the early storage period but declined slightly thereafter. Ethylene treatment did not appear to have any significant effect in increasing or decreasing the concentration over that found in untreated lots after ripening.

STARCH.—Starch was hydrolyzed rapidly during storage. Although microchemical tests showed this material evenly distributed throughout the

TABLE IV  
EFFECT OF ETHYLENE ON CHEMICAL CHANGES OF BARTLETT PEARS RIPENED AFTER STORAGE AT 31° F.

YEARS OF AGE	NO. DAYS RIPENED	TOTAL SUGARS		REDUCING SUGARS		SUCROSE		SOLUBLE PECTIN		INSOLUBLE PECTOPECTIN		STARCH		ALCOHOL-IN-SOLUBLE RESIDUE		ACID	
		UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED	TREATED
.....	0	7.65	7.65	6.79	6.79	0.82	0.82	0.10	0.10	0.78	0.78	0.99	0.99	4.33	4.33	0.19	0.19
.....	8	9.10	9.20	7.54	7.81	1.48	1.32	0.39	0.57	0.48	0.21	0.30	0.21	3.00	3.06	0.23	0.24
.....	0	8.22	8.22	6.72	6.72	1.43	1.43	0.10	0.10	0.61	0.61	0.68	0.68	3.58	3.58	0.19	0.19
.....	8	9.15	9.15	7.52	7.46	1.54	1.61	0.53	0.59	0.24	0.17	0.27	0.13	3.23	3.15	0.19	0.19
.....	0	8.73	8.73	7.56	7.56	1.11	1.11	0.18	0.18	0.73	0.73	0.41	0.41	3.45	3.45	0.22	0.22
.....	8	8.74	8.96	7.20	7.43	1.46	1.46	0.61	0.69	0.18	0.16	0.23	0.17	3.49	3.53	0.21	0.20
.....	0	8.33	8.33	7.34	7.34	0.94	0.94	0.09	0.09	0.87	0.87	0.19	0.19	3.64	3.64	0.18	0.18
.....	8	9.07	9.11	7.60	7.62	1.40	1.42	0.71	0.79	0.45	0.41	0.16	0.19	3.48	3.57	0.21	0.22
.....	0	8.87	8.87	7.92	7.92	0.90	0.90	0.11	0.11	0.75	0.75	0.15	0.15	3.71	3.71	0.20	0.20
.....	8	9.07	9.03	7.41	7.53	1.57	1.43	0.65	0.64	0.14	0.12	0.15	0.14	3.42	3.42	0.18	0.20
.....	0	8.97	8.97	7.92	7.92	0.99	0.99	0.36	0.36	0.37	0.37	0.08	0.08	3.37	3.37	0.21	0.21
.....	8	9.00	9.03	7.41	7.53	1.51	1.43	0.36	0.37	0.34	0.32	0.07	0.09	2.78	3.00	0.19	0.20



tissues of the fruit at the time of harvest, only traces could be detected after 30 days of cold storage. The hydrolysis of starch, therefore, does not enter into the ripening changes of pears withdrawn after delayed periods of storage. This fact has been observed by MARTIN (22).

During ripening the rate of hydrolysis tended to be more rapid in the treated lots, but starch also disappeared rapidly in the untreated fruit, and the differences observed, therefore, were not as large as those found in newly-picked fruit.

**ALCOHOL-INSOLUBLE RESIDUE.**—The alcohol-insoluble residue decreased throughout the storage period, and no significant differences were found in the amount of this material remaining in treated and untreated lots after ripening. This would be expected since starch was hydrolyzed almost as rapidly in the untreated as in the treated lots. Other undetermined hydrolyzable materials, such as hemicellulose, would probably respond in a similar manner.

**ACID.**—The changes in acid during ripening of the storage fruit were very small, and no significant differences were observed between treated and untreated samples.

**CHANGES IN PECTIC SUBSTANCES.**—Ethylene continued to affect pectic changes for a longer period of time than starch hydrolysis or any other chemical changes observed. In the initial storage sample, protopectin disappeared twice as fast in the treated fruit, and the increase in soluble pectin was almost twice as great. In fruit which had been held for various periods in storage, the rate of protopectin transformation in untreated fruit was more rapid than that observed in pears ripened before storage, and this rate could be increased but little by ethylene treatment. After 30 days of storage, however, slightly more soluble pectin was found in the treated fruit when ripened.

The decrease in effect of ethylene treatment in relation to the increase in maturity of fruit has been observed by ALLEN (1). He found that this gas was more effective in influencing sugar, acid, and starch changes in Gravenstein apples collected early in the season than in those picked at later dates. With pears he also found that the rate of softening and ripening was affected mostly in fruit treated prior to storage. Chemical analysis made after the fruit had been held in storage for 10 and 15 weeks showed no differences in acid or in total and reducing sugars between treated and untreated lots. The suggestion was made, however, that in view of the results on softening and color, it would appear that greater differences in chemical composition might have been found had samples been analyzed a short time after harvesting. The results obtained in the present experiments show clearly that this assumption was correct.

## EXPERIMENTS WITH ANJOU PEARS RIPENED AFTER STORAGE

Some studies of the Anjou pear were included because this variety keeps longer and, as shown formerly (12), responds to ethylene treatment for a longer period of time after storage than the Bartlett pear. The fruit for this series was held at 31° F., and samples were withdrawn for analysis after 20, 60, and 120 days. In addition to chemical analysis of treated and untreated lots, respiration determinations were made during ripening, using the method of HARDING and MANEY (13). Pressure tests before and after ripening-

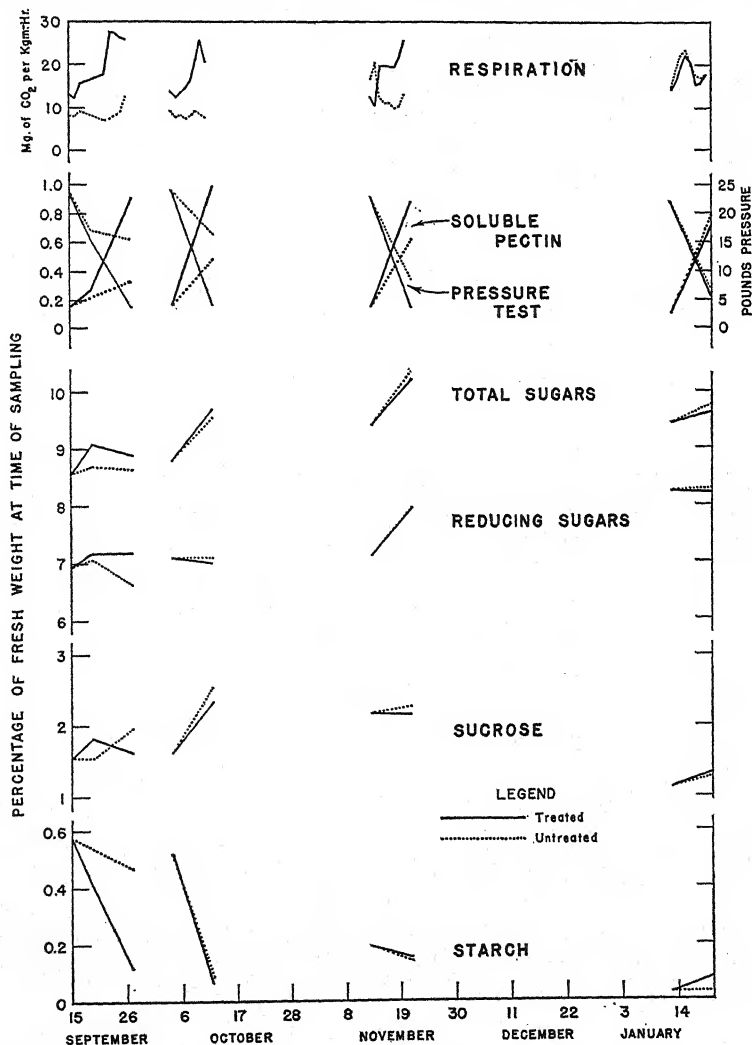


FIG. 2. Effect of ethylene on chemical changes of Anjou pears ripened before and after storage at 31° F.

ing also were made, using the Oregon pressure tester (23). The data presented in table V show the comparative chemical composition and the pressure tests of the ethylene-treated and untreated lots before and after ripening. Comparative changes in sugar, starch, pectin, pressure test, and respiration are represented graphically in figure 2.

The results obtained with the Anjou are similar to those found for the Bartlett, and therefore, will not be discussed in detail.

**CHEMICAL CHANGES.**—The greatest effects from ethylene were obtained early in the storage period. Total and reducing sugars increased more rapidly in the treated lot ripened immediately after harvest; but fruit ripened after being held for various periods at 31° F. showed no differences which could not be attributed to experimental error. In one or two instances sucrose was found to be slightly lower in the treated lots but in most cases the differences were not significant. Since the hydrolysis of sucrose does not occur in the early stages of ripening, as shown by MARTIN (22), it is possible that had the ripening process been observed over a longer period, ethylene might have shown more effect on sucrose hydrolysis. This appears likely, since HIBBARD (16) has reported that he found less sucrose in several kinds of fruits as a result of ethylene treatment.

Starch disappeared more rapidly in the treated fruit ripened at time of storage, but ethylene had little effect upon increasing the rate of its hydrolysis after the pears had been held at a low temperature for even short periods of time.

In the Anjou pear, as in the Bartlett, the pectic changes were influenced by ethylene treatment for a longer period of time than starch hydrolysis or any other chemical change observed. As can be noted in table V, starch hydrolysis was not affected by ethylene even after only 20 days' storage, although there was still considerable of this material in the fruit tissues at this time. Decidedly higher amounts of soluble pectin, however, developed in the treated fruit ripened at this period, and also in the treated lot ripened 40 days later. The rate of pectic changes, though, was eventually unaffected by ethylene, as shown by similar amounts of soluble pectin found in the untreated and treated fruit ripened after 120 days' storage.

**PRESSURE TEST.**—The pressure test decreased only slightly during 120 days of storage, indicating that very little softening of the tissues had occurred while the fruit was held at 31° F. Pressure tests of the ethylene-treated lots were much lower than those of the untreated fruit ripened before and after 20 and 60 days of storage, indicating a much more rapid softening of the tissues as a result of treatment. Treated and untreated fruit which was ripened after 120 days' storage showed very little difference in pressure test.

It is apparent from these results that softening of the tissues and the development of soluble pectin are closely correlated. This is indicated by



the fact that higher pectin content was always associated with lower pressure tests in the ethylene-treated fruit as compared to lower pectin content and higher pressure tests in the untreated fruit. This correlation is also indicated by the fact that fruit ripened under treatment after 120 days of storage showed no increase in soluble pectin over that found in untreated fruit; neither did this fruit show any significant decrease in pressure test as a result of similar treatment.

**RESPIRATION.**—Increases in respiration were obtained long after starch and sugar changes ceased to be affected by ethylene. As can be observed in figure 2, rate of respiration was consistently higher in the ethylene-treated lots ripened before and after 20 and 60 days' storage; but no significant differences were observed between treated and untreated fruit ripened after being held at 31° F. for 120 days. It will be recalled that the development of soluble pectin was also no longer affected by ethylene after a similar storage period, and it is therefore apparent that respiration and pectic changes cease to respond to ethylene treatment at approximately the same time.

Considerable importance has been attributed by KIDD and WEST (18) to the increase in respiration occurring prior to ripening in apples. They have applied the term "climacteric" to this respiratory increase, since they consider that it marks a transition to senescence in the life of the fruit. Ripening of the fruit, *i.e.*, softening of the tissue, the development of flavor and aroma, are considered senescent phenomena; since they were observed not to occur in apples until the climacteric had passed.

Various theories have been advanced to account for this increase in respiration observed to take place at a definite period in the life of the fruit. KIDD and WEST (17) suggested it was associated with a decrease in acidity; WARDLAW and LEONARD (25) to an increase in the internal concentration of oxygen; GUSTAFSON (11) to a decrease in hydrogen-ion concentration. The results of the present experiments indicate that the increase in respiration in pears is not associated with a particular concentration of any specific chemical substance, especially sugars. Neither does it appear to be associated with any change in titratable acidity. The fact that this increase in respiratory activity can be initiated so readily with ethylene would indicate that the basic changes brought about by this gas are undoubtedly causal factors of this phenomenon. In addition, the fact that ethylene is so effective in initiating ripening changes other than the climacteric indicate that possibly the basic changes occurring in all cases may be similar in nature. For this reason, further experiments with ethylene, especially in relation to enzyme reactions, appear justified.

### Discussion

It is assumed from the results obtained in these investigations with pears that ethylene treatment applied at certain periods in the life of the fruit

definitely affects the principal reactions associated with the ripening processes. This is shown by the increased rate of starch hydrolysis, the higher sugar content, the more rapid transformation of protopectin to pectin and by the increase in respiratory activity in the fruit to which ethylene has been applied. The initiation of these chemical ripening processes could be brought about by ethylene treatment of pears picked at a very immature stage, long before the fruit had naturally developed to the period when ripening would normally occur. In addition, the reactions occurring in the presence of ethylene were identical to the changes that were observed to take place in mature fruit ripened naturally under normal conditions. In the light of these facts, the question is raised as to what would be the effects of a natural accumulation of ethylene in the tissues of the fruit.

The magnitude of the chemical changes affected by ethylene during ripening is determined by the maturity of the fruit and by the length of time it is held in storage prior to treatment. As previously pointed out, pears picked while still in an early stage of development ripened readily when subjected to ethylene, while similar fruit, not so treated, failed to ripen altogether or was markedly delayed in ripening beyond the period required for treated fruit. In pears collected at more mature stages, the ripening changes naturally progressed at a more rapid rate, and the effect observed from ethylene, therefore, was less pronounced. After the pears had been held in storage for comparatively short periods of time, the chemical changes resulting in ripening occurred very rapidly when the fruit was removed to a higher temperature, and little if any benefit could be observed from the use of ethylene.

The length of time during which the individual chemical changes associated with ripening are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic transformations and respiration. Starch disappears rapidly after picking or storage, and the hydrolysis of this material does not enter into the ripening process of pears withdrawn after long periods of storage. Pectic changes and respiratory activity cease to respond to ethylene at approximately the same period.

### Summary

1. Ethylene treatment, when applied at certain periods, was found to increase the rate of starch digestion, the concentration of total and reducing sugars, and the transformation of protopectin to pectin in Bartlett and Anjou pears. No changes in titratable acidity were found.
2. The increase in rate of softening observed in pears treated with ethylene is definitely correlated with an increase in rate of pectic changes.
3. The magnitude of the response obtained with ethylene is determined by the maturity of the fruit and by the length of time held in storage prior to treatment.

4. The length of time during which the individual chemical ripening changes are influenced by ethylene varies greatly, being shortest with sugar and starch changes, and longest with pectic reactions and respiration.

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# GROWTH BEHAVIOR OF ONE-MILLIMETER EXCISED ROOT TIPS

GLADYS C. GALLIGAR

(WITH ONE FIGURE)

## Introduction

In 1893 RECHINGER (5) attempted to find the degree of divisibility of plant parts beyond which no growth could take place. He concluded that the presence of vascular strands is necessary to the growth of isolated plant tissue. In 1922 KOTTE (7) refuted this conclusion, which had been corroborated by LAMPRECHT in 1918, by securing as many as six centimeters of growth from one millimeter root tips (including the cap). Since then WHITE (11) has found that the terminal one tenth millimeter detached from a root retains the capacity to differentiate into regions of root cap, epidermis, cortex, and vascular strand. The present investigation was undertaken more for comparison with previous work of the author, in which the original length of the root tips was 10 mm., rather than as a confirmation of KOTTE's work.

## Materials and methods

The procedure used to secure sterile root tips has been outlined in previous papers (4, 5).

Root tips, one millimeter in length, excised from seedlings of dent corn, sunflower, sweet corn, cotton, Gradus pea, and Burpee's Extra Early pea were included in the first series of experiments. The growth of the root tips of cotton and the two varieties of pea was so poor (table I) that these plants were discontinued in subsequent series.

The nutrient solution was a modification of Pfeffer's formula to which dextrose and peptone were added.<sup>1</sup> Immediately after it was made, the

<sup>1</sup> Ca(NO <sub>3</sub> ) <sub>2</sub>	2.0 gm.	KCl	0.25 gm.	Dextrose	2.0 per cent.
KH <sub>2</sub> PO <sub>4</sub>	0.5 gm.	MgSO <sub>4</sub>	0.5 gm.	Peptone	0.04 per cent.
KNO <sub>3</sub>	0.5 gm.	FeCl <sub>3</sub>	0.005 gm.	Distilled H <sub>2</sub> O	6000 cc.

solution was measured into the flasks, which were closed with cotton plugs and autoclaved at 15 pounds pressure for 20 minutes.

## Discussion of results

The data of table I show marked irregularities of elongation, a condition found also in 10-mm. root tips (4). There are no smooth curves of growth, but rather a series of irregular peaks representing spurts of growth at various times. Within the first ten days sunflower and corn grew almost equally

**TABLE I**  
**SAMPLE SERIES OF FIVE INDIVIDUALS OF EACH SPECIES SHOWING DAILY INCREMENTS OF GROWTH IN MILLIMETERS FOR EACH INDIVIDUAL DURING THE MEASURABLE STAGE**

Burpee's Extra Early pea										
1	0	0.5	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0.5	0	0.5	0	0	0	0	0	0	0
4	0	0.5	0	0	0	0	0	0	0	0
5	0.5	0.5	0	0	0	0	0	0	0	0
Sweet corn										
1	0	0.5	0	0.5	0.5	2	1	2	2	0
2	0	0.5	0.5	0	1	0	1	1	1	2
3	0	0.5	1	0	0.5	1	1	1	1	2
4	0	0.5	1	0.5	1	1	2	2	2	2
5	0.5	0.5	0.5	1.5	1.5	1.5	2.5	2	2	1
Corn										
1	1	0	0	0	0.5	0.5	3	7	3	5
2	0.5	0	0.5	0	0	0.5	1.5	2	2	6
3	1	0	0	0.5	0.5	1	2	4	3	2
4	0.5	0	0.5	0	1	1	5	8	5	7
5	1	0	0.5	0.5	4	3	5	7	8	4
Sunflower										
1	0.5	0	0.5	1	0	1	0	1	1	1
2	0	0.5	0.5	1	0	1.5	0.5	1	2	3
3	1.5	0.5	2	2	1	2	3	4	3	1
4	0	0	0.5	0	0.5	0	0	0	0	0
5	0	0	0.5	1.5	0	0.5	1.5	1	1	3
Cotton										
1	0.5	0.5	0	0	0	0	0	0	0	0
2	1	0	0	0.5	0	0	0	0	0	0
3	0.5	5	0	0	0	0	0	0	0	0
4	0	0.5	0.5	0	0.5	1.5	0	0	0	0
5	0.5	0.5	0.5	0	0	0	0	0	0	0
Gradus pea										
1	0	0	1	0	0	0	0	0	0	0
2	0	0	0	1	0	0	0	0	0	0
3	0	0	0.5	0	0	0	0	0	0	0
4	0	0	0	1	0	0	0	0	0	0
5	0	0	0.5	0.5	0	0	0	0	0	0

well while sweet corn was not far behind. But insignificant elongation occurred in cotton, Gradus pea, and Burpee's Extra Early pea.

As a rule the daily rate of growth for the first few days was very slow as compared with that of the 10-mm. root tips of the same species. A faster growth rate, approaching that of the 10-mm. root tips, was attained from the seventh to the tenth day. The slow initial growth may be accounted for by the delayed recovery from the injury of excision. In a 10-mm. root tip the ratio of wounded tissue to the whole surface of the root fragment is insignificant when compared to that of a 1-mm. root tip. In the latter case the wounded area is quite large in relation to the whole surface of the root. The wound is also in a region of high metabolic activity, where the shock resulting from such a wound is much greater than the shock from a wound several millimeters removed from the actively growing meristem. Consequently a slower growth rate may be expected from the shorter fragments during the first few days. Another factor causing more rapid initial growth in the longer fragments may be the greater store of natural reserves of food in their mature tissues.

The data on sunflower, dent corn, and sweet corn are summarized in table II. These figures offer opportunity for interesting comparisons with the results of the 10-mm. root tips (4). All three varieties of root tips cut at 1 mm. are alike in having average total gains in length within the first ten days that fall far short of the corresponding gains for roots having an initial length of 10 mm. One-millimeter root tips of dent corn and sweet corn also fall short in the figures on final total length, range in total length, average number of secondary roots, range in number of secondary roots, and average dry weight for the 10-mm. root tips. Strangely enough, final data on 1-mm. sunflower root tips exceeded those of 10-mm. root tips, showing that sunflower performed much better unencumbered with several millimeters of mature tissue in initial stages. This is diametrically opposed to RECHINGER's view (5) that differentiated vascular tissue is essential to the growth of isolated plant parts.

A comparison of 1-mm. root tips of the different species with each other discloses some interesting facts (table II). The data on the average values for sweet corn were lowest throughout except in the case of dry weight, which was slightly greater than that of corn, indicating that sweet corn was more able to attain its growth in length at less expense of its own reserves. The reverse was true when the original lengths of sweet corn were 10 mm. (4). Sunflower was superior to corn and sweet corn in every respect except in average increment in length the first ten days and in average number of branches. The appearance of secondary roots occurred from the thirteenth to the sixteenth day in dent corn, sweet corn and sunflower,

TABLE II

A SUMMARY OF THE DATA ON 105 ROOT TIPS CUT AT ONE MILLIMETER, INCLUDING THE ROOT CAP, AND GROWN IN THE DARK IN THE MODIFIED PFEFFER'S SOLUTION PLUS 2 PER CENT. DEXTROSE AND 0.04 PER CENT. PEPTONE

SPECIES	AV. DAILY INCREASE IN MM. OF LENGTH OF 30 ROOT TIPS FOR FIRST 10 DAYS										AV. TOTAL LENGTH AT END OF 10 DAYS	AV. FINAL TOTAL LENGTH	RANGE IN FINAL LENGTHS AMONG INDIVIDUALS	FINAL AV. NO. LATERAL ROOTS	RANGE IN FINAL NO. LATERAL ROOTS AMONG INDIVIDUALS	AV. DRY WT. PER 10 ROOTS
	1	2	3	4	5	6	7	8	9	10						
Sunflower ..	mm. 0.55	mm. 0.63	mm. 0.54	mm. 0.59	mm. 0.67	mm. 1.68	mm. 1.25	mm. 1.79	mm. 1.20	mm. 1.42	mm. 10.32	mm. 121	mm. 43-283	35	0-89	gm. 0.283
Sweet corn ..	0.33	0.30	0.43	0.85	0.15	0.94	1.32	2.00	1.60	1.61	9.53	85	33-155	13	0-69	0.078
Corn .....	0.40	0.25	0.31	0.45	0.88	0.85	1.92	3.05	2.20	2.50	12.81	89	24-189	38	5-61	0.074

somewhat later than in the longer initial fragments, while Gradus pea, Burpee's Extra Early pea and cotton never produced laterals. Several one millimeter fragments of corn, sunflower, and sweet corn appeared to cease growth for as long as twelve to fifteen days, after which growth was resumed

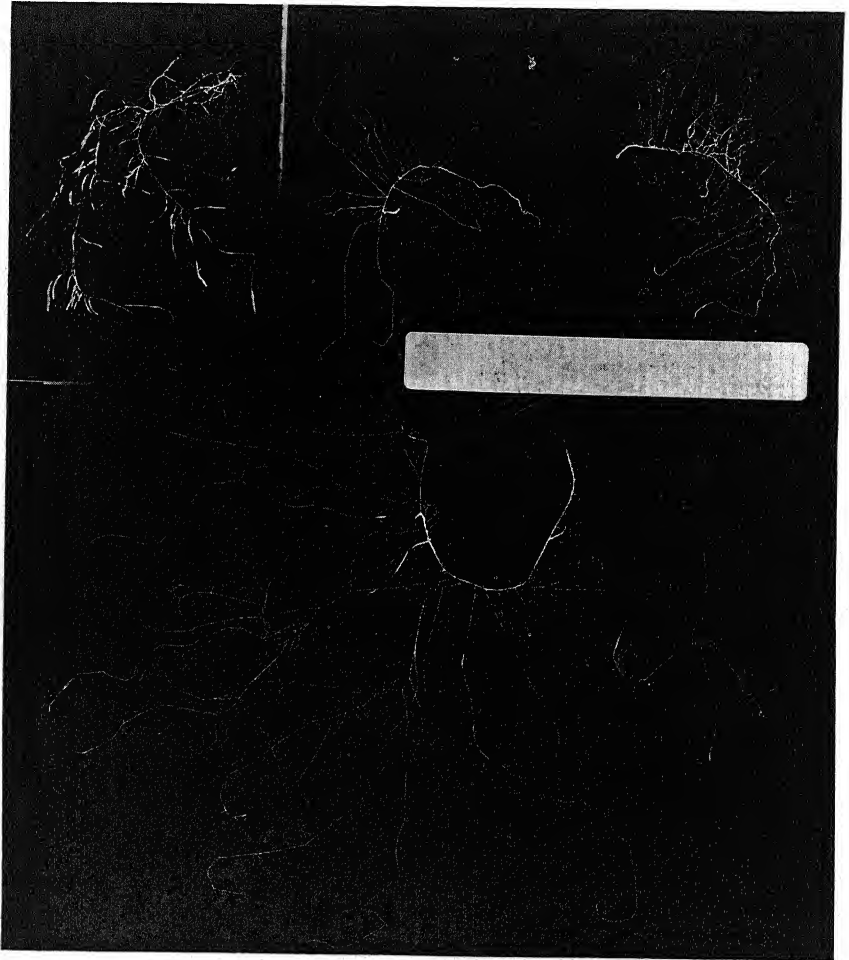


FIG. 1. Growth habits of root tips cut off at 1 mm. and allowed to grow undisturbed in Pfeffer's solution plus glucose and peptone.

Upper left, sunflower root after four months. Note the usual papillae; root hairs are visible near the growing tips.

Upper right, four sweet corn roots after four months. Note the corkscrew twist in the two small ones.

Lower center, a single corn root after four months. Note the extremely fine and ramifying threadlike lateral roots.

or secondaries were produced which grew to great length. This phenomenon was most marked in sunflower, but was also noticeable in corn and sweet corn.

As may be seen in figure 1 each species exhibited certain peculiarities of its own during the process of growth. From 40–50 per cent. of the root tips of dent corn manifested a tendency toward the development of a very short main root axis and a very decided development of laterals. Figure 1 shows a corn root 157 mm. in length with 54 secondary roots, nine of which range from 104–217 mm. in length. This single individual is sufficient evidence that excised root tip meristem of 1-mm. length, including the root cap and devoid of demonstrable differentiated vascular tissue, is capable of enormous growth and development.

On the other hand sweet corn manifested a strong tendency toward development of the main axis of the root and suppression of laterals. About 40 per cent. of the roots developed no laterals at all; about 10 per cent. produced laterals profusely; the rest developed only a few short laterals. Often the roots of sweet corn grew in tight corkscrew spirals (fig. 1), never attaining any great length in such cases. This behavior may have been caused by an injury in excision to one segment of the meristem initials. Occasionally from such spiral roots very fine thread-like laterals grew to lengths of 15 mm. or more (fig. 1).

Except for an initial slower rate of elongation, the 1-mm. tips of sunflower behaved like the 10-mm. tips (4). In a few root tips a curious hypertrophy of cells occurred, in which there was no normal elongation, but merely a proliferation of a mass of undifferentiated parenchyma-like cells, similar to the growths secured by WHITE (11) on the integumental cells of the seed primordia of *Antirrhinum majus*. The cell masses attained the size of a large pea. The individual cells seemed not to be firmly held together as in ordinary parenchyma.

### Summary

1. There was no regularity of growth in excised root tips having an original length of 1 mm.
2. The daily rate of elongation within the first ten days was much slower than that of root tips cut at 10 mm.
3. Within the first ten days sunflower and corn surpassed sweet corn in rapidity of elongation, while cotton, Gradus pea, and Burpee's Extra Early pea grew scarcely at all.
4. Corn showed a tendency to develop a short root with an abundance of laterals, while sweet corn manifested the reverse condition.
5. The presence of vascular tissue was not necessary to the growth of excised root tips.

6. One-millimeter sunflower root tips achieved greater growth than 10-mm root tips.

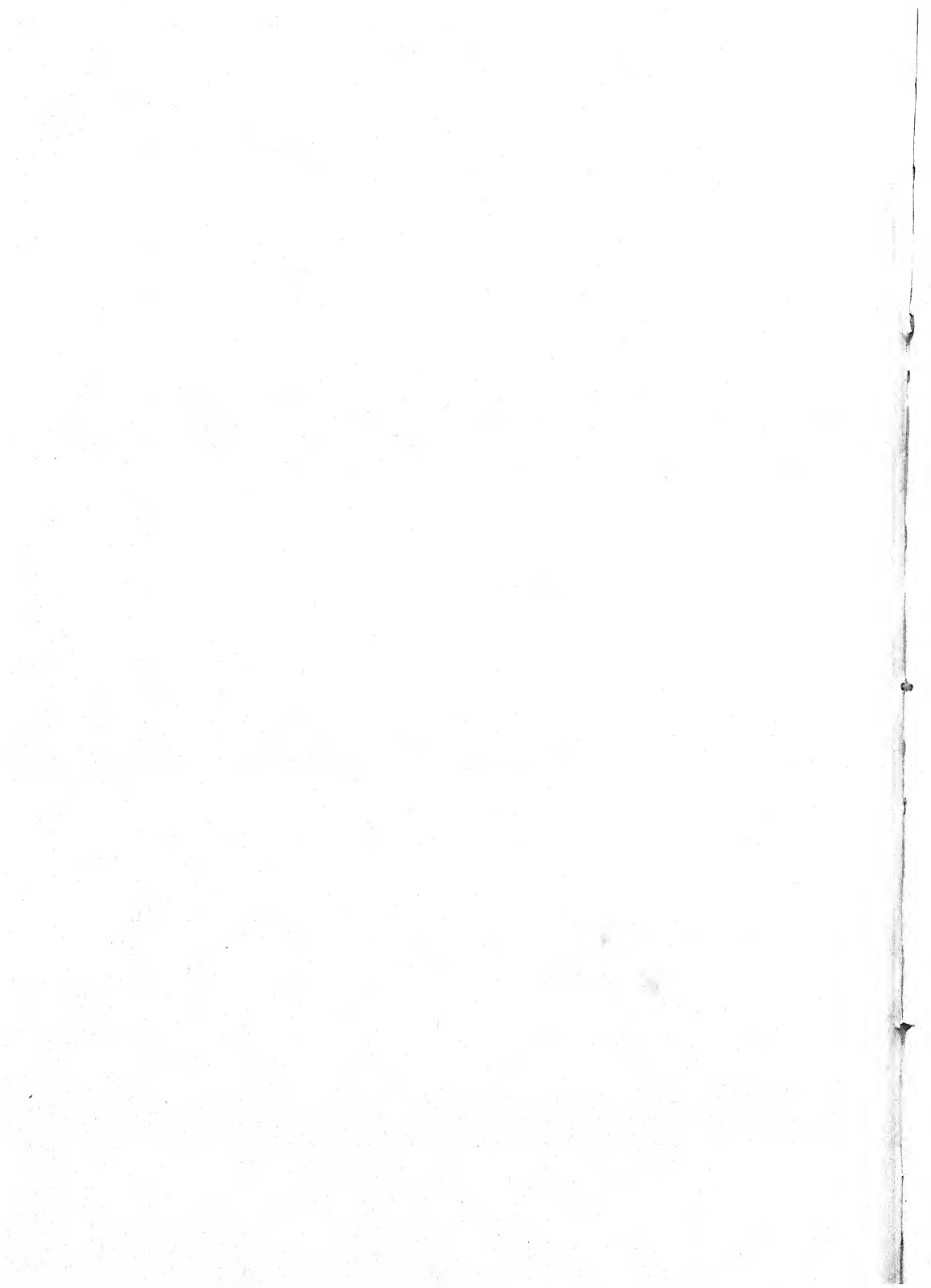
7. Corn root tips were less able to accumulate dry weight when cut at 1 mm. than when cut at 10 mm.

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# TRANSPIRATION AND THE ABSORPTION OF MINERAL SALTS<sup>1</sup>

KENNETH E. WRIGHT

## Introduction

The effect of varying rates of transpiration upon the absorption of mineral salts is an old question that has been studied by numerous investigators. No attempt will be made here to review these bulky data. Earlier opinions, as summarized by CURTIS (2), were usually in favor of increased absorption of mineral salts. More recent studies by various investigators are characterized by a decided lack of unanimity of opinion. The work of HASSELBRING (7), KIESSELBACH (9), MUENSCHER (12), MENDIOLA (10) and others indicates that different rates of transpiration are without effect upon the absorption of mineral salts. Investigations conducted by HASS and REED (6), HITCHCOCK and ZIMMERMAN (8), and FREELAND (3, 4) support the contention that transpiration is a factor which affects the absorption and translocation of mineral salts.

The confusion that exists in this field of investigation is probably caused largely by the failure to eliminate all other factors in the metabolism of the plant, which may affect the absorption of mineral salts. It is the purpose of this paper to present a technique that will predominantly keep these other contributing factors constant, and, with such controlled conditions, to demonstrate that different rates of transpiration are accompanied by corresponding rates in the absorption of certain anions and cations.

## Methods

Seeds of *Phaseolus vulgaris*, var. *humilis*, bush bean, were germinated in distilled water and grown to the flowering stage in HARTWELL and PEMBER's (5) culture solution. Six plants were then transferred to a round battery jar which had a disc cut from ply board to fit the top, with holes bored through it to receive the plants. The wooden disc was paraffined to prevent water loss. A wide overlapping rubber band held the paraffined disc on the top of the jar, and cotton plugs maintained the plants in position. The interior of the jar was kept dark by a coating of black asphalt paint over the outside surface of the jar. Each of the several jars was filled with 2000 cc. of culture solution. The entire system was then weighed, and allowed to stand in the greenhouse for ten days. At the end of this period all of the jars were again weighed. Two were selected that had lost the same weight of water measured in grams. These jars were subsequently

<sup>1</sup> Published by permission of the Director of Research, as contribution no. 525 of the Rhode Island Agricultural Experiment Station.

filled with 2000 cc. of culture solution and placed in a high and low humidity cabinet.

This cabinet was made of window glass, except the wooden floor and the framework, and was divided into two compartments by a glass partition. In one chamber humidity was kept high by means of a pan of water, and by water vapor given off by the transpiring plants. In the other chamber a low humidity was obtained by connecting this chamber in a circuit with a Frigidaire freezing unit, and forcing air through the circuit with an air pump driven by an electric motor. The air pump was located in the cold air portion of the circuit between the freezing unit and the cabinet. By the time the cold air had gone through the pump and the remaining circuit its temperature was the same as that of the high humidity chamber. This circuit was a closed system, thus eliminating the possibility of a fresh supply of carbon dioxide accelerating the rate of photosynthesis with a consequent increase in the supply of sugars to the root system. The volume of the humid chamber was 8.06 cu. ft., and that of the dry chamber, because of the additional space in the freezing unit, was 8.78 cu. ft. This difference was considered sufficiently small to have little significance in affecting the sugar supply, and consequently the absorption capacity of the roots.

Circulating air, as opposed to still air, should have no effect on the aeration of the culture solutions, as the containers were airtight with the exception of compact cotton plugs which held the plants in position in the paraffined top of the container.

The cabinet was placed in the greenhouse with a cloth screen in position between the cabinet and the direct rays of the sun to prevent the temperature inside the cabinet from rising above that of the greenhouse.

The plants were in the cabinet for ninety-six hours, and then the remaining culture solution in each jar was measured and retained for analysis. The jars were refilled with 2000 cc. of culture solution and were replaced in the cabinet for another run, but in this second run the plants that had previously been in the high humidity chamber were placed in the low humidity chamber. Those that had been in the low humidity side of the cabinet were placed in the high humidity side. At the conclusion of a ninety-six hour run the residual culture solutions were again measured and saved for analysis.

The original culture solution and the residual culture solutions were analyzed for calcium and for nitrates according to the standard methods as described by the Association of Official Agricultural Chemists (1). Phosphorus was determined by the colorimetric method of TRUOG and MEYER (13), and potassium by the colorimetric method as described by MORRIS and GERDEL (11).

### Results and discussion

The results as recorded in table I are typical of two other runs made with different sets of plants at different times of the year. An examination

TABLE I

AMOUNT OF MINERAL ABSORPTION WITH HIGH TRANSPIRATION AND LOW TRANSPIRATION

TREATMENT	WATER ABSORBED	PHOS- PHORUS ABSORBED	CALCIUM ABSORBED	NITRATES ABSORBED	POTAS- SIUM ABSORBED
	<i>cc.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Jar 1—high transpiration ...	330	13.6	25.0	41.4	35.6
Jar 2—low transpiration .....	150	8.6	15.0	41.0	27.8
Jar 2—high transpiration ...	335	11.2	27.0	46.8	56.4
Jar 1—low transpiration .....	165	9.6	13.0	41.8	52.8

of the table demonstrates that in all cases a higher rate of transpiration is accompanied by an increased absorption of the various ions. It is admitted that in any one specific instance the difference in absorption of a certain ion under conditions of high transpiration and of low transpiration is insignificant. However, it is emphasized that a summation of all the analytical evidence illustrates a definite trend favoring increased absorption of minerals under conditions of increased transpiration.

Selecting for experimentation two jars of plants that in the greenhouse lost the same amount of water over a 10 day period, infers a root-shoot balance with approximately the same rate of absorption in both jars of plants. Reversing the positions of the plants in the chambers of the cabinet serves to counteract the effect of varying metabolic processes associated with different sets of plants. With such a procedure it was found that the plants in the high transpiration chamber continued to absorb a greater amount of minerals, which indicates that transpiration is evidently a correlated factor in determining the amount of minerals absorbed.

It is believed that the data here presented substantially support the contention that transpiration is a contributing factor in determining the rate of absorption of mineral ions. The writer does not wish to promulgate the idea that transpiration has a function in the absorption of soil minerals, since, given sufficient minerals in the soil, the laws of diffusion would insure adequate mineral absorption without transpiration. However, it is logical to assume from the data here recorded that increased mineral absorption may be the result of increased transpiration.

### Summary

The effect of transpiration upon the absorption of mineral ions was determined by analyzing culture solutions in which bean plants<sup>2</sup> had been

<sup>2</sup> The plants were grown in the culture solutions by WALTER COLVIN and VLADIMIR SHUTAK.

growing under conditions of high and low transpiration. The contributing effects of the metabolism of the plants upon mineral absorption were offset by: (1) selecting plants with the same transpiring power when in the same environment; (2) analyzing the residual culture solutions of the same plants under conditions of high and low transpiration; and (3) devising an experimental procedure attempting to make transpiration the only variable. Analytical results indicate that an increase in the rate of transpiration is associated with a corresponding increase in the absorption of certain ions.

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## BRIEF PAPERS

### TIME AND TEMPERATURE OF PROTOPLASMIC COAGULATION<sup>1</sup>

HENRY T. NORTHEN AND REBECCA TYSON NORTHEN

In most plants protoplasmic activity cannot be maintained at temperatures much above 40° C. The cessation of many vital phenomena at temperatures above or slightly below 40° C. is probably caused by the coagulation of the protoplasm. The data presented will show that temperatures of 43.5° C., and above, cause a decided increase in protoplasmic consistency, which has been interpreted as coagulation, in cells of corn, oat, rye, and wheat coleoptiles and that in *Spirogyra* and *Zygnema* a lower temperature effects a decided increase in protoplasmic firmness.

Filaments of *Spirogyra* and *Zygnema* and one week old corn, oat, rye, and wheat seedlings were immersed for various periods of time in tap water maintained at various temperatures. After the desired immersions the plants were transferred to water at room temperature where they were allowed to remain for twenty minutes. The root systems of the monocots were then removed and the shoots and filaments were centrifuged with an acceleration of 680 X gravity for one minute. After centrifugation the filaments and the coleoptiles were mounted in aceto-carmin and the respective number of cells in which the chloroplasts had been moved by the acceleration was determined. In the coleoptiles the subepidermal cells near the tip and adjacent to the veins were selected for study.

The data are summarized in table I.

In the table when two time figures (e.g., 6-8) are given the actual time necessary to cause coagulation, as evidenced by no displacement in 90 per cent. of the cells, lies between the two values. When only one figure is recorded it indicates the time necessary to cause coagulation in 90 per cent. of the cells.

It is probable that *Spirogyra* no. 1 and *Spirogyra* no. 2 are the same species. Vegetatively they looked alike. *Spirogyra* no. 1 was collected in April when part of the pond was still covered with ice and when the temperature of the water was 7° C., whereas no. 2 was collected in June when the temperature was 18° C. It will be noted that no. 1 coagulated at a much lower temperature than no. 2 and that at 43° C. less than one minute was required to cause coagulation in 90 per cent. of the cells of no. 1, whereas between six and twelve minutes were required to cause coagulation in 90 per

<sup>1</sup> Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 168.

TABLE I

TIME AND TEMPERATURE REQUIRED FOR PROTOPLASMIC COAGULATION

PLANT	TEMPERATURE	MINUTES IMMERSION NECESSARY TO PREVENT CENTRIFUGAL ACCEL- ERATION FROM DISPLACING THE CHLOROPLASTS IN 90% OF THE CELLS
	0° C.	minutes
<i>Spirogyra</i> no. 1 .....	43.0	< 1
	40.0	< 1
	38.5	< 1
	36.0	2
	33.5	30
<i>Spirogyra</i> no. 2 .....	43.0	6-12
	38.0	*(15%)
<i>Zygnema</i> sp. ....	43.0	< 1
	40.0	2
	38.5	4
	36.0	12
	33.5	*(26%)
Corn .....	47.5	6-8
	43.5	30
	40.0	*(0%)
Oats .....	47.5	1-2
	43.5	15
	40.0	*(0%)
Rye .....	47.5	2-4
	43.5	15-20
	40.0	*(0%)
Wheat .....	47.5	2-4
	43.5	12-15
	40.0	*(0%)

\* Coagulation had not occurred in most cells after an immersion of forty minutes. The percentages of cells in which coagulation had occurred, as evidenced by a failure of centrifugal acceleration to displace the chloroplasts, are shown in parentheses.

cent. of no. 2. If they were the same species, which seems likely, then the temperature at which they were growing greatly affected the time and temperature of coagulation. The *Zygnema* was collected with *Spirogyra* no. 1 and it also coagulated at a comparatively low temperature.

Furthermore, the data indicate that at temperatures of 43.5° C., and above, the protoplasm in cells of corn, oat, rye, and wheat coleoptiles coagulates. The time necessary to cause coagulation at 43.5° C. and 47.5° C. suggests that the protoplasm in coleoptile cells of wheat, rye, and oats is more sensitive to high temperature than is the protoplasm in coleoptile cells of corn.

## A METHOD FOR THE TREATMENT OF CUTTINGS AND ROOTS OF THE PECAN WITH ROOT-INDUCING CHEMICALS

C. L. SMITH AND L. D. ROMBERG

In preliminary experiments in the rooting of pecan root cuttings it was found that both water solutions and lanolin mixtures of indolebutyric acid were effective in inducing root formation. It was also found that lanolin mixtures of the chemical applied to the bark of root cuttings larger than  $\frac{1}{2}$  inch in diameter were less effective in inducing root formation than applications made in slits through the bark or in holes bored laterally into the roots. This was apparently due to the inability of the indolebutyric acid to diffuse through the thick bark. In the large roots it was indicated that a localized treatment was more effective than where the entire base of the cutting was treated as by immersing in a water solution. Preliminary work in 1937 also indicated that the indolebutyric acid was effective in inducing root formation in transplanted pecan trees when it was applied in lanolin mixtures in slits or holes in the roots at the time of transplanting.

Since the large root cuttings responded better to localized treatments, and since it was impractical to use water solutions in the treatment of the roots of transplanted trees, the lanolin mixture of indolebutyric acid was applied in holes or slits in the roots by means of a hypodermic syringe. This method was effective but was not satisfactory because of the difficulty of filling the small holes with the lanolin mixture, and because of the waste of material. A method was devised, therefore, which eliminates both these difficulties and also saves time in the operation.

Ordinary round wooden toothpicks are used as the carriers for the chemical instead of lanolin. The indolebutyric acid is dissolved in 95 per cent. ethyl alcohol in the concentration desired. The two ends of each toothpick are cut off leaving the pick 4 cm. long. Then the picks are immersed in the alcoholic solution for 24 hours, after which they are removed and the alcohol allowed to evaporate. Each pick absorbs approximately 0.084 ml. of the solution, and any desired concentration may be obtained by properly adjusting the concentration of the chemical in the alcohol. The picks also absorb approximately the same volume of water as of alcohol, and at approximately the same rate. The chemical, therefore, readily diffuses from the toothpicks when they are placed in the roots. Holes  $\frac{7}{64}$  inch in diameter are bored transversely about two-thirds through the cuttings or roots at the desired points by means of an electric drill, and treated toothpicks of sufficient length to fill the holes are inserted.

The treated picks have been as effective as the lanolin mixture in inducing root formation in cuttings, and have been effective in concentrations vary-



ing from 0.25 to 4 mg. per pick; but it appears that the concentration of 4 mg. per pick is too high, since some injury has occurred from its use. Further work is being done on concentrations, on effectiveness of the treatments on the rooting of cuttings, and on the reestablishment of the root systems of transplanted pecan trees.

BUREAU OF PLANT INDUSTRY

U. S. DEPARTMENT OF AGRICULTURE

AUSTIN, TEXAS

PLANT PHYSIOLOGY IN THE WOMEN'S COLLEGES<sup>1</sup>

DOROTHY DAY

A composite picture of the women's colleges shows that each offers one or two courses in plant physiology, generally only one in the physiology of the higher plants. Such courses are usually for juniors and seniors although sophomores or graduate students may be included. They may meet for one-half year or one year, but this in no way indicates that more or longer courses are any better than one short, well-taught course.

Classes in plant physiology are relatively small, often about four students, partly because of the keen competition of the social sciences and liberal arts. Laboratory space is not a factor governing the type of work, although planning seems to be required in some cases. Lack of apparatus becomes a problem only when it is complex or expensive. Individual work is preferred, especially for simpler problems, but often two students to an experiment is necessary to conserve apparatus. May it not be that two students paired wisely, perhaps a chemist and botanist as Dr. LYON suggested last year, might stimulate each other to good advantage? Although stock solutions may be prepared by the instructor or an assistant, it is considered advisable for the student to make some of them, as emphasis on laboratory technique and cleanliness is training that reflects on the college after graduation.

In the recording of experiments variation is great, but there is a hint of informality rather than adherence to rigid specifications. There is clear emphasis, however, on accurate recording of observations with proper discussion or explanation. Student results do not always agree with theory. This may mean that students work until results are obtained which check with accepted theory, or more often they are required to account, if possible, for the deviation. Sometimes this helps them to understand how their inadequate technique may cause errors. Too often they have high aspirations which are never realized because of their inexperience. Errors from lack of practice and from poor technique may blast their high hopes but may also bring a greater appreciation of fine critical research.

The living plant is used more than physical and chemical illustrations. Prerequisites emphasize the botanical background more than the chemical, and perhaps do this to a greater extent than in the large universities. They include elementary botany, plus a second course in botany in several cases. Chemistry as a prerequisite varies through all degrees from none at all to the advice that "some knowledge of organic chemistry is desirable"; or a

<sup>1</sup> Summarized from the report at the Richmond Meeting of the American Society of Plant Physiologists, December 30, 1938.

year of chemistry or physics may be recommended. The advanced course requires more preparation, usually the elementary physiology plus more botany or chemistry or both. By this scheme of greater emphasis on the botanical background for physiology, the graduates of our women's colleges come to the university well prepared for advanced work and later take important positions in plant physiology.

Support for physiology is mostly from majors in the botany department, not from students in horticulture, for there is no graduate or professional training school to lend strength. Physiology is not required for the major in botany in more than one or two colleges. It is difficult for girls in the various other sciences to meet all the prerequisites. Physiology is not regularly elected by students of horticulture, as they generally lack even elementary chemistry and are usually more interested in the art aspect of landscaping. Several instructors in horticulture and in landscape architecture in these colleges have expressed interest in physiology and have indicated that they felt each course might be stronger if the alliance were closer. This is a different situation from that of the land-grant colleges where so many of the students in horticulture take botany, chemistry, and physiology, and then go into some commercial branch of agriculture—the practical growing of apples, roses, potatoes, or Christmas trees, or the investigations connected with them.

Apparently teachers keep in mind new aspects of physiology, introduce them into the laboratory if this seems advisable, or at least present them briefly in the lecture. In some cases lack of time compels use of not too complicated and well-tried experiments. Standard experiments seem to be the rule, at least for basic problems. Added to the general assignment, there may be latitude in the choice of one or more of several experiments in certain fields according to interest and ability. The girls who ask for problems in which they do not know the answer have ample opportunity to satisfy their healthy curiosity with the aid of some direction from the teacher.

Since it is impossible to cover the subject of plant physiology thoroughly in one-half year or even a year, the trend is toward inclusion of the major phases so that a student has at least heard of them, and toward emphasis on teaching the *student well* rather than the *subject thoroughly*. By teaching a student to think, we may open her eyes to see more clearly what is going on in the world about her. Years ago they said that our New England farms were exhausted, and now they say that the west is being depleted; but China has survived for forty centuries and more. Agriculture and plant physiology in our country are young, and so it is good for the youth of the nation to recognize such problems and to plan well in advance. Agriculture is a basic industry, and the growing of plants may mean the

life or death of a nation; in fact, the growing of plants in America may mean the life or death of this democracy. Lead the student to consider not one isolated process in the laboratory, but to think in terms of the living plant and of a permanent democracy.

DEPARTMENT OF BOTANY

SMITH COLLEGE

NORTHAMPTON, MASSACHUSETTS



## NOTES

**Richmond Meeting.**—The fifteenth annual meeting of the American Society of Plant Physiologists, held at Richmond, Virginia, December 28 to 30, 1938, was made an occasion of quiet but sincere celebration of fifteen years of successful constructive progress. The programs were arranged in parallel sessions, in cooperation with the Physiological Section of the Botanical Society of America, whose officers in the finest spirit agreed to a pooling of papers so that those on a single topic might be arranged in non-conflicting programs. This is the first time that such an arrangement has been found feasible, and it was so successful that we hope this enlightened policy may be followed on future occasions.

The annual dinner on Wednesday evening was featured by the retiring president's address, and by the usual announcements of awards, and election of a corresponding member. Dr. CURTIS's address, *Education by authority or for authority?* was appreciated as a challenge to educational policy.

Joint sessions were held with the Botanical Society of America, the American Society for Horticultural Science, and the American Phytopathological Society.

On Friday evening a symposium on the teaching of plant physiology was held as the final session, with the leadership of Dr. BURTON E. LIVINGSTON of the Johns Hopkins University. Two formal and two informal addresses made up the program. A large audience remained for the meeting, and it is obvious that earnest thinking is going on in regard to the teaching problems in this field. In response to this obvious interest in the educational aspects of our work, a standing committee of the society has been appointed to give some permanence and opportunity for more consistent planning of the programs. Taken as a whole the entire meeting was well planned. Much credit for the success of the Richmond meeting goes to the officers of the society, and especially to Dr. W. R. ROBBINS, program committee chairman, and his efficient aids, Dr. H. H. ZIMMERLEY, Dr. D. G. CLARK, and Dr. F. P. CULLINAN, *ex-officio* member of the committee.

**Hales Award.**—The sixth recipient of the STEPHEN HALES award is Dr. JOHN WESLEY SHIVE, who has served for many years as plant physiologist at the New Jersey Agricultural Experiment Station, and professor of plant physiology at Rutgers University. He went to the Station in 1915, and is nearing a quarter of a century of distinguished service in that institution.

Dr. SHIVE was born at Halifax, Pennsylvania, February 13, 1877. His first degrees, Ph.B. and A.M., were obtained at Dickinson College in 1906 and 1907. In 1913 he went to the Johns Hopkins University where he enjoyed a

fellowship for two years, and received his Ph.D. in 1915. During this period he worked on the salt nutrition of plants, out of which work came the three-salt nutrient solutions which, with modifications, have been very widely used among plant physiologists. From Hopkins he went to the New Jersey Station, and has been a great leader in the field of salt requirements of agricultural plants, and general plant nutrition. The citation accompanying the award by Dr. WALTER F. LOEHWING, president of the Society, is as follows:

"In recognition of a life-time of distinguished service to plant physiology, the American Society of Plant Physiologists now confers the STEPHEN HALES Prize for 1938 on JOHN WESLEY SHIVE.

"For nearly twenty-five years associated with the investigation of the mineral requirements of plants; originator of new and of improved methods for the growth of plants in culture under controlled conditions; one of the earliest to appreciate the fundamental relationship of the hydrogen-ion activity of the culture solution to the absorption of the inorganic ions by the plant and to develop practical methods of plant culture based upon these relationships; student of the chemical composition of plant tissues as affected by the nature of the culture solution; inspiring teacher and leader of a devoted corps of younger investigators; a man whom this Society is proud to have among its membership and a scientist whose single-mindedness of purpose and definiteness of aim have led him to achievement of the highest caliber."

**Barnes Award.**—Each year since 1926 the American Society of Plant Physiologists has elected one or more of its members to a CHARLES REID BARNES life membership in the Society. Every fifth award is made to some distinguished physiologist from foreign lands. The fifteenth award, made at the Richmond meeting, went to Professor Dr. LUDWIG JOST, who for so many years has distinguished the chair of plant physiology at Heidelberg. JOST is not only distinguished for his research. Plant Physiologists all over the world owe him a debt of gratitude for his contributions to the teaching of plant physiology through his text-books, which have been models of excellence. He followed, in this regard, in the footsteps of SACHS and PFEFFER. The award carries with it the good wishes and high regard of American plant physiologists generally.

**Corresponding Member.**—One corresponding member was elected at the Richmond meeting. This honor was bestowed upon Sir E. JOHN RUSSELL, distinguished director of the Rothamsted Experimental Station, and of the Imperial Bureau of Soil Science, at Harpenden, England. RUSSELL is best known among plant physiologists for his outstanding work, *Soil Conditions and Plant Growth*, the best single work on this subject in the English language. The Society is honored to have him among its group of distinguished corresponding members.

**Corporate Seal.**—In July 1937, announcement was made at the Denver meeting of the adoption of a design for a corporate seal for the American Society of Plant Physiologists. In fulfillment of an expressed intention to make public the design adopted, we are reproducing it here. The design is readily understood, and needs no explanatory remarks. It emphasizes the

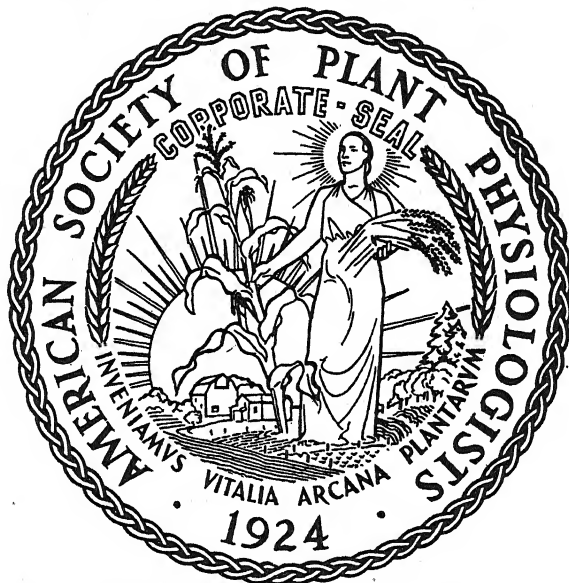


FIG. 1. The corporate seal of the American Society of Plant Physiologists.

practical value of fundamental research dealing with the processes of plant life. The founding of the Society in 1924 was followed by corporate existence in 1932. The adoption of a seal met a government requirement in connection with the revenue laws.

**Milwaukee Meeting.**—The summer meetings of the American Society of Plant Physiologists have become almost as interesting and valuable as the midwinter meetings, and as well attended. The season is usually appropriate for driving. The next meeting will be held at Milwaukee, Wisconsin, during the week of June 19 to 24, 1939. The chairman of the program committee for this meeting is Dr. W. E. TOTTINGHAM, one of the past presidents of the Society. Working with him are Dean I. L. BALDWIN, and Dr. L. F. GRABER, all of the University of Wisconsin. Announcements will be made at the proper time through the mails, as to the exact dates, hotel arrangements, and the meetings. Excellent highways and railroad connections to Milwaukee will make transportation a simple matter. Milwaukee is famous; its hospitality for these meetings will be appreciated and enjoyed by all who come. Is it unanimous? Excellent intellectual fare is in store for every one.



**Program Committee.**—In anticipation of the meeting at Columbus in December, 1939, President LOEHWING has appointed Dr. B. S. MEYER, Department of Botany, Ohio State University, Columbus, Ohio as chairman of the program committee for the sixteenth annual meeting. Dr. J. D. SAYRE of the Ohio Agricultural Experiment Station at Wooster has also been appointed to this committee. Those who may be planning to report before the Society at that time may find it advisable to have their titles ready early. The constantly increasing number of papers has made parallel programs advisable, and those who reply most promptly will be most easily accommodated with time. Fuller reports will be made in later numbers of PLANT PHYSIOLOGY.

**Patrons.**—During the year 1938 two of our members joined the group of patrons of the Society. They are Dr. WALTER F. LOEHWING, of the State University of Iowa, and Dr. HERMAN A. SPOEHR, of the Carnegie Institution of Washington. This increase of patronage is very gratifying, and is an evidence of the deep interest our members are taking in the problems of permanent support for the Society's program. There are now seven patrons. To all of those who have so generously supported the financial needs of the Society we record the deep appreciation of the entire membership. With the progress already made we hope it is not too much to look forward to other additions to the growing list of supporters. The editor once said, and now repeats that there is "no more enduring benefit to mankind which one can leave behind him than the possibility of continuously increasing knowledge." The need for funds to support our program of research and publication was never greater than at the present moment. And the old Scotch proverb that "Mony a mickle makes a muckle" applies to the continuous growth of our funds. We trust that many friends may wish to share in the realization of a permanent financial foundation for the advancement of plant physiology as a science. Plant physiologists have a way of doing the things they really wish to see accomplished.

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**John Henry Schaffner.**—On January 27, 1939, Professor JOHN H. SCHAFFNER, long time professor of botany at Ohio State University, died at Columbus, Ohio. Professor SCHAFFNER was in his seventy-third year, having been born at Agosta, Ohio, July 8, 1866. He received his degrees from Baker (A.B. 1893), and the University of Michigan (A.M. 1894). He was a graduate student at the University of Chicago in 1896–1897, and spent a year at Zürich in 1907–1908. He was professor of natural science at South Dakota preceding his student days at Chicago, joined the faculty of Ohio State University in 1897, and there spent 40 years in teaching and research. For 10 years he was head of the department of botany (1908–

1918). During this period, in 1916, he married CORDELIA GARBER of Mt. Vernon, Ohio. They have three children. Since 1918 he has been Research Professor of Botany at Ohio. He was editor-in-chief of the Ohio Naturalist from 1900 to 1915, and of the Ohio Journal of Science from 1915 to 1917. He was president of the Ohio Academy of Science, 1908-1909, and was a fellow and member of many scientific organizations. He published a number of botanical books, and contributed many papers to science journals. His special field of interest lay in the field of sex and sex reversal among plants. He was specially interested in the cases in which he thought sex was not controlled by chromosome mechanisms.

The science of botany has lost a devoted research man, and the American Society of Plant Physiologists one of its distinguished members. We deeply regret his passing, and extend our sympathy to his bereaved family.

**Plant Physiology.**—The second edition of Dr. EDWARD C. MILLER's well known *Plant Physiology* was issued by the McGraw-Hill Book Co. late in 1938. The work has been carefully revised, and many additions made to the material presented in the first edition. It is a monumental volume of 1201 pages. Dr. MILLER has accomplished a task few would be willing to undertake, and he has given us a work which will be of great value for years to come. It is the American equivalent of the great volumes such as SACHS, PFEFFER, and JOST gave to the world. The entire field is covered in 14 chapters, and the author has done remarkably well in summarizing the vast stores of published work. A rough estimate indicates that more than three thousand different authors have been consulted. It is an indispensable volume for both student and instructor, a source of valuable aid in research. Dr. MILLER deserves the thanks of all plant physiologists for his unselfish service, and praise for having accomplished his task in admirable manner. The quoted price of the work is \$7.50, and orders may be sent to the McGraw-Hill Book Co., New York.

**Plant Physiology.**—The second edition of the English translation of N. A. MAXIMOV's text-book has been published under the title *Plant Physiology*; it is edited by Dr. R. B. HARVEY and Dr. A. E. MURNEEK from a translation of the fifth Russian edition prepared by Dr. IRENE V. KRASSOVSKY. The arrangement has been changed from the earlier edition, and MAXIMOV rewrote much of it because of the great advances made since the earlier text was published. With the additions of the data and viewpoints of American investigators by the editors, it is a new book, and has been given a distinctive title to prevent confusion with the former translation.

The presentation begins with the physico-chemical organization of plants, and their chemical composition and general metabolism. Chapters on res-

piration, and growth, precede those on carbon and nitrogen assimilation. Then the author turns to mineral nutrition, water relations, and translocation of substances within the plant body. The later chapters discuss such topics as resistance to cold and drought, correlations, physiology of development, processes connected with flowering and fruiting, and ripening of fruits and seeds. The final chapter takes up seasonal phenomena such as dormancy and the breaking of dormancy. The sequence is somewhat unusual, and illustrates that various arrangements of a body of facts may be equally acceptable as a guide to study.

This book, popular in its first edition, will probably be even more popular in its second. The situation as regards texts has changed somewhat, as there is now competition between many meritorious works. The Russian plant physiologists have been very active investigators, and Dr. MAXIMOV has been one of the ablest leaders of the Russian school.

It is a boon to American students to have the rich resources of Russian workers made available in this manner. The book contains 473 pages, 144 text figures, and may be obtained from the publishers, the McGraw-Hill Book Co., at \$4.50 per copy.

**Structure of Economic Plants.**—An excellent contribution to the literature of developmental anatomy is this work by Professor HERMAN E. HAYWARD of the Department of Botany, the University of Chicago. The work is divided into two parts, the first of which deals with the general anatomy of plants. The four chapters covering this general field are entitled: cells and tissues and their development; the anatomy of the root; the anatomy of the shoot; and the anatomy of the flower and fruit.

The second part presents detailed studies of the developmental anatomy of certain crop plants. Sixteen different crop plants have been chosen for this exposition, from Gramineae to Compositae. The plants chosen are corn, wheat, onion, hemp, beet, radish, alfalfa, pea, flax, cotton, celery, sweet potato, white potato, tomato, squash, and lettuce. A bibliography is provided for each chapter, and there is a helpful glossary of anatomical terms at the end of the volume. The book is profusely illustrated, 340 figures, and the illustrations are beautifully reproduced. In every way the book is a fine example of book making. The text is written in very clear and readable style, and the material presented will prove extremely valuable to plant physiologists. When anyone works on any kind of plant he should know its anatomy as thoroughly as is exemplified in the treatment of the plants chosen for this text. That is asking a great deal, but not too much, on the anatomical side. The list price of this indispensable work is \$4.90 per copy. It is published by the Macmillan Co., New York, to whom orders may be sent.

**Physiology of Plants.**—Among the new textbooks of Plant Physiology we welcome the appearance of *The Physiology of Plants* by Dr. WILLIAM SEIFRIZ of the University of Pennsylvania. It is a truly elementary work, written simply and clearly, but with enough detail to give the student a wide introduction to the field of plant physiology. There are 26 chapters, and they are relatively brief, the whole work comprising, with index, only 314 pages. The arrangement of subject matter follows a logical order, and the work seems to be very well adapted to the needs of student and teacher. The book is published by John Wiley and Sons, New York, and the price is \$3.50 per copy.

**General Plant Physiology.**—In the July, 1938 number of PLANT PHYSIOLOGY we announced the publication of *General Plant Physiology*, by E. C. BARTON WRIGHT, who is well known to American readers. At the time, it was stated that an American publisher might be announced later. We are happy to call attention to the fact that this work has now been printed in the United States, so that it is readily available for use in our laboratories and class rooms. Orders may be sent to P. Blakiston's Son and Co., Philadelphia. The price for the American printing is \$4.50 per copy.

**Ascorbic Acid.**—An interesting and valuable monograph entitled *L'Acide Ascorbique dans la Cellule et les Tissus* is published as volume 16 of the *Protoplasma Monographien*, a series from the press of Gebrüder Borntraeger, Berlin. The author is A. GIROUD of the faculty of medicine, University of Paris. The material is presented in three parts. The first part is a general introduction to our knowledge of ascorbic acid, and to the various methods of research which have been developed for its detection and quantitative estimation. The second part discusses in detail the facts concerning ascorbic acid in animal cells, tissues, and organs; the third part considers the problems of ascorbic acid in plant cells. The distribution of the acid in various parts of the plant body, variations with age, seeds, and germination, mature plants, physiological considerations, relations to the chlorophyll function, relationship to sugars, and the influence of ascorbic acid on growth. The several bibliographies list about 450 titles. The publication is timely, and brings together a wide spread literature. The quoted price is RM 12 for cloth bound copies. Orders may be addressed to Gebrüder Borntraeger, W 35 Koester Ufer 17, Berlin, Germany.

**Advances in the Chemistry of Natural Organic Substances.**—The press of Julius Springer, Wien, Germany, has issued the first volume of an annual, *Fortschritte der Chemie organischer Naturstoffe*, which is a collection of summaries in certain fields of plant biochemistry. The editor is L. ZECHMEISTER, Pécs, with the collaboration of A. BUTENANDT, F. KÖGL, W.

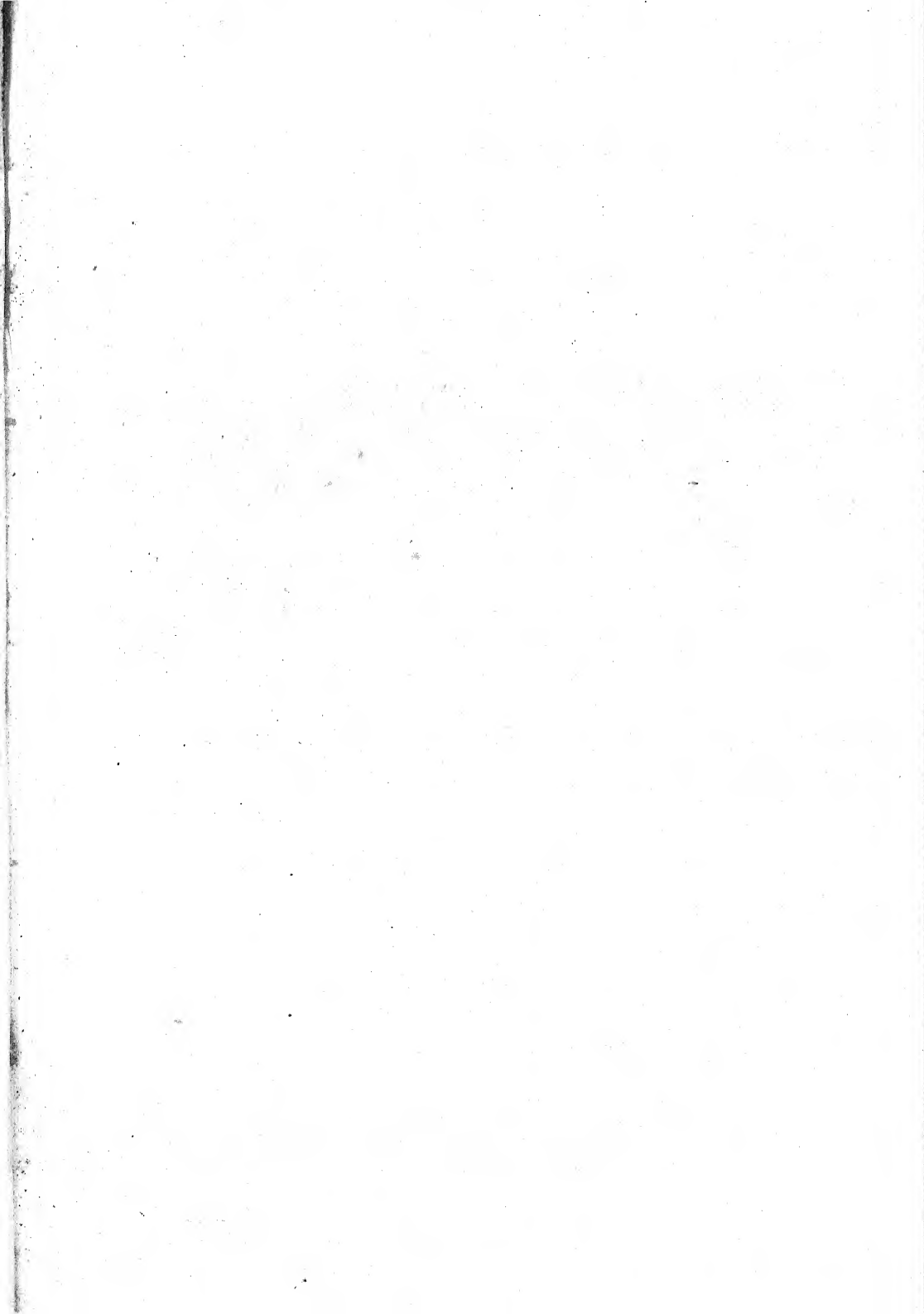
N. HAWORTH, and E. SPÄTH. The high standing of these keen investigators is a guarantee that this annual will present an unusually valuable series of reviews. The volume for 1938 contains seven reports. The first deals with the newer methods for glucoside syntheses, prepared by G. ZEMPLÉN. It includes alkylglucoside syntheses by chemical means, biochemical syntheses, syntheses from acetohalogen compounds, transformations of  $\beta$  to  $\alpha$  forms, mercury salt methods, preparation of biosides of the  $\alpha$  series without the employment of acetohalogen compounds, and phenol-glucoside- or phenol-bioside-syntheses by HELFERICH's method with the aid of  $\text{ZnCl}_2$  or p-toluol-sulphonic acid.

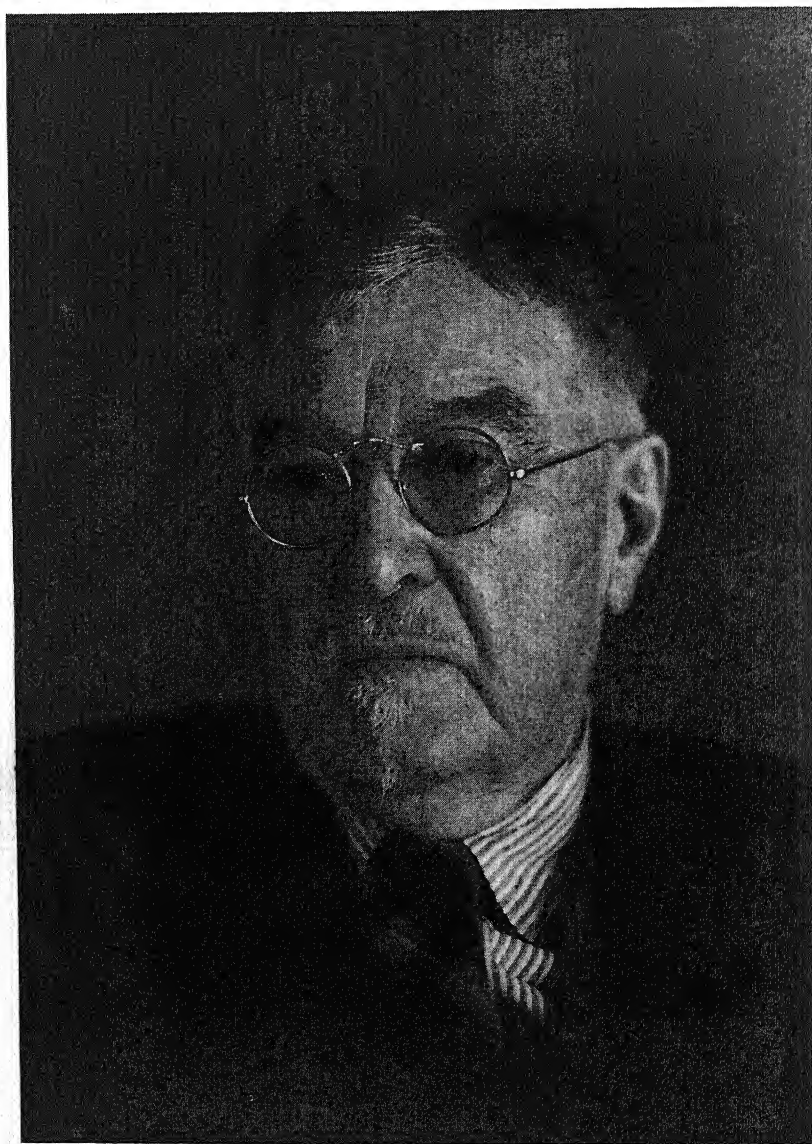
The succeeding reviews deal with the following topics: The component glycerides of vegetable fats, by T. P. HILDITCH; recent advances in the chemistry of the sterols, by I. M. HEILBRON and F. S. SPRING; cozymase, by H. VON EULER; nucleic acids, by H. BREDERECK; chlorophyll, by A. STOLL and E. WIEDEMANN; and the application of physical methods to the study of natural substances: form and size of dispersed molecules: roentgenography, by O. KRATKY and H. MARK.

All of them are valuable monographs on the recent work in the fields mentioned. The work is illustrated with 41 text figures, and is provided with extensive author and subject indexes. Every student of the biochemical processes of plants and animals will welcome these authoritative accounts of the recent accomplishments of biochemists. A complete file of this work will be needed in every library, and on the desks of the workers. Orders should be addressed to Julius Springer, Wien (Vienna).

**Poisonous Plants.**—Under the title *Poisonous Plants of the United States* Dr. W. C. MUENSCHER of Cornell provides an interesting and informative book on the poisonous vascular plants of the United States. About 400 species are included in the discussion, and they are classified according to the families in which they occur (ENGLER system). Sixty-eight families contain toxic members, and 75 well drawn figures will aid in identification of some of the commonest offenders. In the first section some interesting groups are mentioned. There are almost 100 species listed as causing dermatitis, 12 that have photosensitizing effects on animals, 16 that cause hydrocyanic acid poisoning, 18 that are too strongly seleniferous in regions where selenium is abundant in the soil, 28 that cause undesirable flavors in dairy products, and 18 that are liable to produce mechanical lesions, external or internal, because of spines, awns, hairs, or burs. The work has been very ably done, and the book supplies a long-felt need for comprehensive information in this field.

The publishers are the Macmillan Co., 60 Fifth Ave., New York, to whom orders may be sent. The quoted price is \$3.50 per copy.





DANIEL TREMBLY MACDOUGAL  
From a photograph taken in May, 1938

# PLANT PHYSIOLOGY

APRIL, 1939

DANIEL TREMBLY MACDOUGAL

PIONEER PLANT PHYSIOLOGIST\*

(WITH ONE PLATE AND TWO FIGURES)

It was about fifty years ago and at DePauw University, where he was at that time a junior student assistant, that the young DAN MACDOUGAL undertook, with only a window-sill for a laboratory, to follow some of Professor J. C. ARTHUR's typewritten directions for experiments in plant physiology. He was apparently successful, for the next year found him at Purdue University, working as instructor in Professor ARTHUR's laboratory. With Professor ARTHUR's encouragement—and in spite of the admonitions of one or two others, who thought there was "no future for a young man in plant physiology"—he continued to show more interest in physiology than in other aspects of botany. After three years at Purdue, he moved on to take charge of his chosen subject at the University of Minnesota, where he remained till 1899.

With leave of absence for 1895-96, MACDOUGAL studied at Leipzig with WILHELM PFEFFER and visited the laboratories of JULIUS SACHS, HERMANN VÖCHTING and other leaders in Germany, Holland and England. Research begun with PFEFFER led to an important contribution on "The Curvature of Roots," on the basis of which Purdue University conferred on MACDOUGAL one of the three Ph.D. degrees ever awarded by that institution to persons not in residence.

Three years after MACDOUGAL's return from abroad he left Minneapolis to become director of the laboratories at the New York Botanical Garden. In that capacity—later as assistant director and at times as acting director of the New York Garden—he contributed much in an administrative way to the fine organization of that institution, but there was no let-up in his al-

\* The American Society of Plant Physiologists dedicates this issue of PLANT PHYSIOLOGY to DANIEL TREMBLY MACDOUGAL, in celebration of the occurrence of his seventy-fifth birthday, on March 16, 1939.—*Committee on the MACDOUGAL Celebration*: FRANCIS E. LLOYD, HOMER L. SHANTZ, CHARLES A. SHULL, BURTON E. LIVINGSTON.



ways vigorous attacks upon fundamental scientific problems. Also he gave generously in helpful suggestion and encouragement to those advanced students who were so fortunate as to spend portions of their formative periods in his laboratories.

It seems that MACDOUGAL had begun to accumulate his great fund of observational knowledge about desert plants and their environments as early as 1891, when he devoted the summer to botanical collecting in Arizona, under the auspices of the U. S. Department of Agriculture. In his years at Minneapolis and at New York he made a number of field excursions to study the plants of the American Southwest. Thus he was ready to give enthusiastic support to H. V. COVILLE's suggestion that the newly founded Carnegie Institution might well undertake special botanical investigation in that region. He served on the board whose recommendations and plans led to the establishment of the Desert Laboratory, near Tucson, in 1903, and it was natural for him to become the first director of the new laboratory as well as of the Department of Botanical Research of the Carnegie Institution of Washington, positions which he held from 1906 to the time of his official retirement in 1928.

At the Desert Laboratory and later also at the Coastal Laboratory—at Carmel, California—MACDOUGAL carried out many long-time studies and experiments with plants in their natural setting, as well as much laboratory experimentation, all in the general field of plant physiology, ecology and plant geography. With a staff of able and enthusiastic colleagues, he developed his department to a high degree of versatility and effectiveness. Visiting investigators were always more than welcome and were furnished with whatever materials and facilities were requisite for what they wished to do. He conducted many excursions into regions of special ecological interest—among the mountains, plains and valleys of Arizona, southern California and northwestern Mexico. In company with GODFREY SYKES, he spent a period in the Libyan desert. Observational knowledge and experience gained in the field were continually brought to bear on physiological experimentation in laboratory, shop and experiment garden. He has turned again and again to problems of plant water relations, of growth and enlargement, of imbibition and swelling. He invented, improved and brought into use several auxographic instruments of precision, the best known of which is the MACDOUGAL dendrograph, an apparatus for the automatic recording of volume changes in tree trunks. By its means he has contributed very greatly to our knowledge of growth in trees. His recent book, on "Tree Growth," summarizes eighteen years of persistent study.

While engaged in all these varied scientific and administrative activities, MACDOUGAL has found much time and energy to devote to the organization of American science workers. For many years he has been active in the

several special societies that deal with plant science, also in the American Association for the Advancement of Science. He was active in the development of the Pacific Division and of the Southwestern Division of the American Association. At the Indianapolis meeting of the American Society of Plant Physiologists (December, 1936) he was elected to a Charles Reid Barnes life membership in that society.

It is quite impossible to sketch adequately, even in faintest outlines, the character and accomplishments of DANIEL TREMBLY MACDOUGAL, whose interests have been so wide, and yet so discriminating, and whose contributions have been so valuable in so many ways. All who know him for his work and all who love him for his genial personality will surely join me in urging him to keep on for many more years, continuing to enlarge and clarify the fields of knowledge which he has already so devotedly and successfully illumined.—GEORGE T. MOORE.

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Although MACDOUGAL was necessarily kept very busy with a large amount of administrative work while at the New York Botanical Garden, he was always ready to discuss botanical problems and research projects with any one of his colleagues or students who wished to undertake or to continue promising investigation. His friendly personality, his thorough general knowledge of botany and his great enthusiasm for research made his New York laboratory, on the upper floor of the Museum Building, in Bronx Park, a very pleasant place in which to work. He set a fine example to all about him, for he was always engaged in scientific projects of his own, from which he generally secured valuable scientific results for subsequent publication. An early riser, he was often seen about the garden, greenhouses and experiment grounds when gardeners and workmen were just beginning their day; also, it was in the early morning that he accomplished much of his writing. Inveterate experimentalist and laboratory worker that he was, he also keenly loved the out-of-doors and liked to devote holidays to short field excursions with a few companions, with whom many things might be discussed by the way. One who held a research scholarship at the New York Botanical Garden at that time loves to recount how he accompanied MACDOUGAL on a memorable tent-camping excursion of several cold and snowy days at the end of November in 1903, to the barrens and marshes inland from Barnegat, on the New Jersey shore.

In his New York period MACDOUGAL published his monograph on the formative influence of light on plants, which gives accounts of many ingenious experiments and a wealth of valuable new observations. When he undertook the important task of editing for publication the California lectures of HUGO DE VRIES on the mutation theory (HUGO DE VRIES. Species

and Varieties, etc. Ed. by DANIEL T. MACDOUGAL. Open Court Publ. Co., Chicago, 1905.), he was already very familiar with many of DE VRIES's new ideas and experimental methods. He had established an experiment garden at the Bronx as early as 1902 and had there been studying mutation in his own cultures of the great evening primrose. That small experiment garden—only about half an acre in extent—was apparently the first in this country to be devoted to studies of this kind. DE VRIES pointed out that this American student had furnished additional proof of the validity of the mutation theory. The publication of these lectures first brought the new theory clearly before English-speaking botanists. In a lecture given before the Barnard Botanical Club, of Columbia University, on December 18, 1905, just before he left the New York Garden for the Desert Laboratory, MACDOUGAL made what appears to have been the earliest announcement of a successful attempt to alter inheritance or induce mutation by means of experimental treatments of plant ovaries. Referring to his injection experiments with *Raimannia* ovaries, he pointed out that "agencies external to the cell may induce mutations, and consequently exert a profound influence on heredity."

Realizing that one of the prime requisites for productive outdoor studies on plant growth and reproduction is definite knowledge about the influential conditions of plant environments, MACDOUGAL installed the requisite instruments at his experiment garden and accumulated for several years detailed records of precipitation, air humidity, air temperature and soil temperature. He was among the first to try to study these environmental conditions quantitatively, especially soil temperature, with reference to plant performance. His keen interest in this climatological aspect of physiological ecology has been maintained throughout his subsequent years of productive investigation. He began to devise and improve climatological and auxanometric recorders of several kinds while at New York, showing a remarkable degree of native ingenuity in that field of research.

From New York MACDOUGAL made a number of brief excursions into the arid Southwest, bringing back plant material and many living plants to be grown in the New York Garden. It was clear to every one at that time that the acute and energetic mind of the director of the laboratories was greatly attracted to problems concerning the ecology and physiology of cacti and other plants of arid climates. His interest in desert plants increased and bore fruit, until, as the leading American authority on desert ecology, he was almost suddenly transplanted from New York to Tucson, to become director of the Desert Laboratory and of the Department of Botanical Research of the Carnegie Institution of Washington.—C. STUART GAGER.

Alone and on foot, with a single burro to carry the packs and with camp equipment consisting of just "a Navajo blanket, a tin cup and a jar of beef extract," MACDOUGAL spent most of the summer of 1891 collecting plants in northern Arizona. The experiences of that summer must have stimulated a native pioneering instinct and traits of alertness, resourcefulness, originality and capacity for prompt decisions, which have distinguished the effective investigator and administrator whom we know. The Flagstaff country was revisited in 1898 and the regions of Tucson, Torres and Guaymas were visited in 1902. He returned to the mountains and valleys about

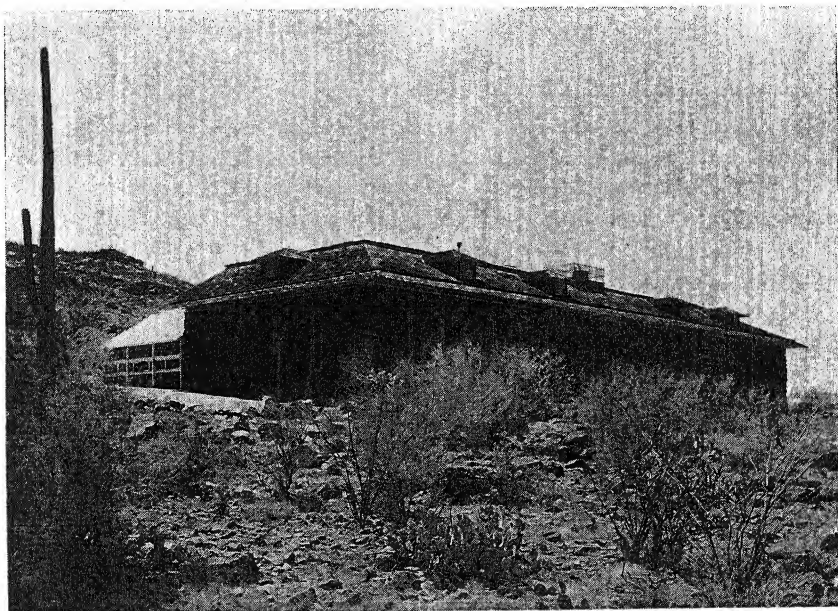


FIG. 1. Main Building of the Desert Laboratory, 1911.

Tucson in 1899, when he and F. V. COVILLE selected the location for the projected Desert Laboratory, on behalf of the recently founded Carnegie Institution of Washington. The first building of the new laboratory was erected in 1903 and MACDOUGAL visited it in the spring of 1904, extending his excursion at that time to the delta of the Colorado River. In the late winter of 1905 he and GODFREY SYKES began their long-continued studies of the delta region. Thereafter he devoted much study to the recently flooded Salton Basin, which he visited at least twice each year for a number of years.

Well equipped with general and special knowledge concerning the plant geography and ecology of the American Southwest, and with unbounded

enthusiasm for further studies in that region, MacDOUGAL became Director of the Department of Botanical Research of the Carnegie Institution of Washington at the beginning of 1906, when he migrated from New York to Tucson and made the Desert Laboratory his official headquarters. The recently erected building, high on a shoulder of Tumamoc Hill, was about three miles away from what was then in many respects a frontier town, and the development of facilities for scientific work involved many difficulties.



FIG. 2. The Director of the Desert Laboratory, 1923.

Transportation was slow, one walked or rode horseback, and goods were brought from town by team. The primitive road and the water supply required immediate attention. A three-room addition and a small greenhouse were promptly added to the original building, and the extensive grounds were fenced. Climatological instruments were soon installed and a small working library was assembled. All such duties and difficulties were im-

mediately faced by the new director with vigor and efficiency, and several important research projects were at once started.

MACDOUGAL's deep and friendly interest in the members of his staff was constantly sustained; he responded generously to all requests for equipment, which were naturally numerous and extremely varied. He liked to have about him fellow workers rather than assistants—staff members and visitors who were not dependent but who told him what they intended to do next. He was generous with helpful suggestion but he never gave commands and there was never a more loyal or more cooperative group than the one he contrived to have about him. He was remarkably successful in engaging the whole-hearted cooperation of many investigators in other laboratories.

The director was habitually first to appear in the morning and he accomplished most of his writing before the rest of us were up. One of his secretaries, remorseful at always finding upon his arrival in the morning that the director was already busily at work, came earlier and earlier for several days, but when he was at length able to reach the office before MACDOUGAL the latter seemed more annoyed than pleased. Routine office work was quickly dispatched but there was always time for a cordial correspondence with other science workers throughout the world. He readily grasped the views of others and his judgments concerning their ideas were always appreciative and friendly as well as keenly critical.

With equal talents for laboratory experiment and field observation and with equal interest in them, MACDOUGAL's first aim seems to have been to assemble and evaluate many widely different kinds of knowledge so as to make possible a true appreciation of plants and their activities as they occur in nature. Now and again he would go away with one or two companions—by saddle and pack train, by team or by automobile—for a day or more of camp life in the nearby mountain ranges or on the great bajadas of the Tucson region. Such enjoyable excursions, pleasantly remembered by many visitors at the Desert Laboratory as well as by staff members, always resulted in new observations and interpretations, and they were recreational in the best sense. It was on field trips of this kind that one discovered one's host to be a grand master of outdoor cookery; he specially liked to broil a steak "the size of a vest-pattern" over a bed of coals.

MACDOUGAL is now residing near Carmel, California, where, freed from administrative responsibility, he is continuing his studies, especially on tree growth, with the same ingenuity and keen enthusiasm that characterized the years before his retirement. His productive activity has shown no let-up, his work shows no signs of being finished, he is the same MACDOUGAL whom we have known these many years.—FORREST SHREVE.

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of

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# CARBOHYDRATE AND RESPIRATORY METABOLISM IN THE ISOLATED STARVING LEAF OF WHEAT

G. KROTKOV

(WITH TWELVE FIGURES)

## Introduction

From the point of view of its respiratory constitution the foliage leaf of the wheat plant is an example of an interesting and somewhat special type of plant organ. The essence of its constitution is the possession of a high potential enzymic activity relative to its low primary reserve of substrate materials, and consequently the wheat leaf passes through its ontogeny rapidly and has a brief duration of life. A leaf of this type contrasts strongly with such organs as potato tubers, or apples, which stand at the opposite extreme, having substrate reserves which are enormous in relation to their potential enzymic activity. As a result such organs maintain themselves for long periods of time and their physiological state changes very slowly.

From numerous observations by different workers on all kinds of plants and detached organs which respire at a rate proportional to their sugar content it has been concluded that sugars serve as the usual respiratory substrate. It must be noted, however, that the great majority of such observations are of short duration and are usually made on material which has just been removed from its natural surroundings. Consequently, such observations apply to respiration in the initial stages of starvation only.

ONSLow, KIDD, and WEST (11) using storage apples as experimental material, followed changes in sugar content and respiration for about 150 days. Over this interval of time, or any part of it, they found that  $\text{CO}_2$  output was roughly equivalent to the total sugar loss. It may be concluded that in apples sugars serve as the respiratory substrate for at least 150 days in storage. Similarly BARKER (1), working with potato tubers in storage, found sugars to serve as the only respiratory substrate. In addition, by exposing tubers to cold for various lengths of time, this investigator brought about variations in the sugar content of tubers between 0.2 per cent. and 7.0 per cent. and then studied the corresponding changes in their respiration. By plotting respiration rates of tubers against their sugar contents he found that the curve so obtained had the form of an "enzymatic rectangular hyperbola," the usual form of relation between the rate of an enzymic reaction *in vitro* and the concentration of its substrate.

It appears then that sugars serve as the respiratory substrate in various plant organs immediately after their separation from the plant, and in organs of certain type, for a long period afterwards. From BARKER's re-

sults one would expect to find the basic relation between respiration rate and sugar to be hyperbolic, but often much obscured by interfering circumstances.

Much less is known of the effects of depletion of this primary substrate on plant metabolism. The fullest account of these effects is found in the work of DELEANO (4). As experimental material this worker used grape vine leaves. On detachment from the plants half of the leaves was used at once and the other half was kept in darkness for various lengths of time. The  $\text{CO}_2$  emitted from these leaves was measured and at the end of the various periods of starvation, leaves were analyzed. It was found that for any interval of time within the first 5 or 6 days all of the  $\text{CO}_2$  produced by the leaves was roughly equivalent to the sugars lost, on the assumption that these sugars were completely broken down into  $\text{CO}_2$  and water. Within this first period there was no appreciable degradation of proteins. After 5 or 6 days proteins began to disappear from the leaves, and the  $\text{CO}_2$  evolved was then in excess of that which could have been derived from the sugars simultaneously lost.

Unfortunately no continuous records of respiration are given in DELEANO's paper, so that it is impossible to say whether this degradation of proteins is correlated with any marked changes in the respiratory rate such as occur in other starving leaves and which are so typical of the respiration record in the first foliage leaf of wheat. After the experiments reported in this paper had been completed a paper was published by YEMM (13), who, working with starving barley leaves, reached the same conclusions as DELEANO as to the nature of the respiratory substrate on prolonged starvation. But the respiration records reported by YEMM are of a different type from those of wheat, and the large hump which is so characteristic of the respiration record of wheat is lacking or highly modified in barley. Clearly then, data on the relation between respiratory activity and the substrate materials on prolonged starvation in organs of the wheat-leaf type are lacking. The experiments reported in the following pages are intended as a contribution toward the rectification of this defect.

### Materials and methods

The data treated in this paper are the results of four experiments in which changes in the rate of  $\text{CO}_2$  emission and changes in the sugars were concurrently determined during the starvation of the isolated, first foliage leaf of *Triticum compactum* Host. variety Little Club. The four populations of seedlings were all grown from the same lot of seed. This was a pure line of Little Club obtained from two sources: the Rust Research Laboratory in Winnipeg, and the Bureau of Plant Industry in Washington. Seed from the two institutions was mixed in the proportion 4 to 9. All the

experiments conducted are numbered in chronological order and will be referred to by number throughout the paper. In each experiment plants were grown in soil until their first leaves reached the stage of "early maturity" at which point a large stock of leaves was detached and used for experimentation.

It has been found by DUFF and FORWARD (5) that, in the course of their metabolic drift with age, wheat leaves grown under standard, artificial conditions enter the stage of early maturity at a little under two weeks from the time of planting. The significant known characteristics of this stage are as follows:

1. The leaves have attained their maximum length.
2. The leaves are active in photosynthesis and the endosperm reserve of the seedling is exhausted.
3. The initial respiration rate when detached, has fallen sharply from high values of the order of 100 mg. of  $\text{CO}_2$  per 100 gm. of leaf fresh weight per hour, which are characteristic of the early stages of growth, and has reached a more nearly stable value of about 35 mg., from which it very gently declines for the several weeks during which the state of maturity is maintained.
4. The leaves contain no starch.

The conditions under which the seedlings were grown before experimentation were different in all the experiments. In experiment 1, the plants were grown under the standard conditions described by DUFF and FORWARD (*loc. cit.*). On the other hand, the plants used for experiments 2, 3, and 4 were grown in the summer in the greenhouse where no attempt was made to regulate external conditions, and where both temperature and light varied considerably. It was not surprising to find that the seedlings grown for the different experiments differed from one another in a number of ways. The most obvious difference was that of their sugar content, which in experiment 1 was several times greater than in experiments 2, 3, and 4. Though there was more similarity among the three populations grown in the greenhouse than there was between any one of them and the plants grown under artificial conditions, nevertheless, plants from the greenhouse were not identical. The sugar content of the leaves in experiments 2 and 4 was nearly the same, but was considerably lower in experiment 3. It would be reasonable then to expect that in plants grown under such different conditions, the physiological state should turn out to be different if appropriate criteria of comparison could be developed.

On the day when the experiment was to begin, after a period of prolonged illumination, the entire population of seedlings was brought to a sufficient degree of homogeneity by removal of the extreme variants with respect to height. From these selected plants the first leaves were detached at the ligule and placed in a beaker with distilled water. The stock of leaves so isolated served as the actual experimental material and from it the fol-

lowing samples were taken: (1) A sample of about 50 or 75 leaves for the respiration determination; (2) a sample of 50 leaves for the first sugar analysis; (3) two samples of 50 leaves each in experiments 2, 3, and 4 for determinations of the changes in fresh weight during starvation. The remainder of the stock of leaves, after taking the above described samples, was set aside standing in water at the temperature of the respiration experiment, and used for subsequent periodic sampling for sugar analyses.

The sample for the respiration experiment was immediately weighed, transferred to the respiration chamber, and the determination of the  $\text{CO}_2$  begun. The sample for sugar analysis, after weighing, was cut at once into small pieces and dropped into boiling alcohol for the extraction of sugars. The initial weights were taken of both samples for the determinations of the leaf fresh weight, and then both samples were placed in two separate beakers with the rest of the stock of leaves, in the dark at the temperature of the respiration chamber, where they were kept till the end of the experiment, their fresh weights being taken daily.

The determinations of respiration rates were extended continuously over the whole period of starvation, which required about 5 days for completion. During the whole of this period the leaves were standing in a glass respiration chamber with a few ml. of distilled water at the bottom. The respiration chamber was kept in darkness in a constant-temperature water bath with temperature variations not exceeding  $0.25^\circ \text{C}$ . for the whole period of starvation. In experiment 1 the temperature of this bath was kept at  $22.3^\circ \text{C}$ . and in experiments 2, 3, and 4 at  $20.0^\circ \text{C}$ . By means of a clock mechanism the current of air, continuously drawn through the respiration chamber, was switched to a new Pettenkoffer tube every 3 hours in experiment 1 and every 4 hours in experiments 2, 3, and 4, so that in each experiment a continuous record of respiration was obtained.

In a wheat leaf, starch can be found only in the early juvenile stage and it disappears later, on entering into the stage of early maturity (5). It has been assumed then that in this last stage no degradation products of starch, such as maltose, can be present. This assumption is fully supported by NEWTON (9) who, working with wheat leaves in various stages of their development, found no complex sugar other than cane sugar. It should be remembered, however, that this worker used leaves which were not strictly comparable with the leaves used in the present work. The writer is not certain that the sugars found in earlier stages of development of the first leaf are necessarily the same as in the later stages. Work is being carried on now on the isolation and identification of the various sugars from wheat leaves and until this is completed no final statement can be made as to the nature of the sugars present. It should be remembered, however, that the leaves dealt with in the present experiments were all in the same stage of their physio-

logical development and consequently would not differ from one another as a result of variation in sugars with age. Moreover, for the present purpose it is not necessary to define exactly what sugars are present. It is sufficient to divide all of the alcohol-soluble carbohydrates of the wheat leaves into two fractions based on their reducing power before and after inversion. In the first fraction, under the name of "simple sugars," are put all those sugars which showed reducing power before acid inversion. In the second fraction, under the name "invert sugars," are included those carbohydrates which showed reducing power after inversion with acid. The amounts of the various carbohydrates present in both fractions have been calculated as glucose. The methods adopted for the extractions of sugars from the leaves and for the subsequent preparation of the alcoholic extracts for the sugar analyses were essentially those of DAVIS, DAISH and SAWYER (3). Into their technique two modifications were introduced. First, in all the experiments the time of extraction has been extended to about 30 hours. This modification has been adopted on the strength of results from several preliminary experiments which have indicated that to remove the bulk of sugars from the leaves it is necessary to carry out extraction for many hours. In one such experiment 13.7 gm. of wheat leaves 12 days old from planting were extracted with four changes of 43 per cent. ethyl alcohol which was made 0.005 N with respect to ammonia. The four alcoholic extracts so obtained were converted into aqueous extracts and finally analysed for their sugar content before and after inversion with HCl. The results of analyses are given in figure 1.

The second modification introduced was that all the inversions of reserve saccharides present in the extracts were carried out not by means of invertase or citric acid, but by means of HCl according to the method adopted by the Association of Official Agricultural Chemists (10).

The procedure, so modified, was then briefly as follows: a sample of leaves was quickly weighed, cut into small pieces, and dropped into 250 ml. of boiling 85 per cent. ethyl alcohol which was 0.01 N with respect to ammonia. After about 30 hr. of boiling in a water bath under reflux condenser, the contents of the extraction flask were transferred to an Erlenmeyer flask, a few ml. of toluene were added, the flask was stoppered tightly and stored in the dark until the preparation of the extract for analysis could be carried further. The extract was then filtered from the leaf residue and the alcohol was evaporated under diminished pressure at a temperature not exceeding 45° C. The residue was taken up in water and cleared with basic lead acetate. The excess of lead was removed as carbonate, the filtrate was made up to volume, adjusting its reaction with NaOH to a value slightly above pH 8. In this form the extract was employed for analysis, its reducing power being determined by the original



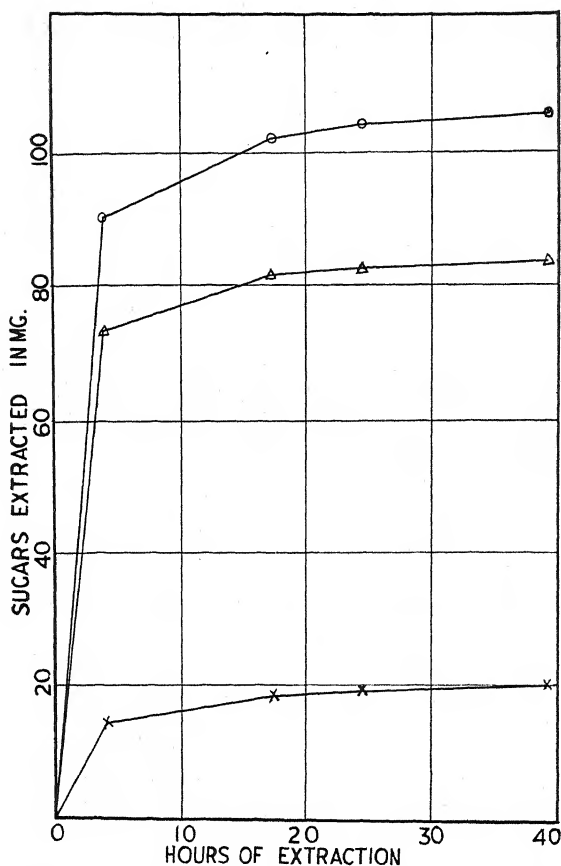


FIG. 1. Absolute amounts of sugars removed from leaves on prolonged extraction  $\times$ — $\times$ — $\times$ , reducing sugars;  $\Delta$ — $\Delta$ — $\Delta$ , invert sugars;  $\circ$ — $\circ$ — $\circ$ , total sugars.

HAGEDORN-JENSEN method (8) before and after inversion with HCl. The actually determined quantities were therefore the reducing sugars (before inversion) and the total sugars (after inversion). The invert sugars were estimated by difference.

### Results

The respiration records of the 4 experiments are given in figures 2 to 5. Experiment 1 was carried out at 22.3° C. and the others at 20.0° C. The records have been plotted without correction for the temperature difference. Temperature corrections have been made wherever comparisons of rates at single temperatures are needed, the temperature-respiration relation having been worked out by DUFF (6) over the range from 10° to 30° C.

The records are all of the striking general form some modification of

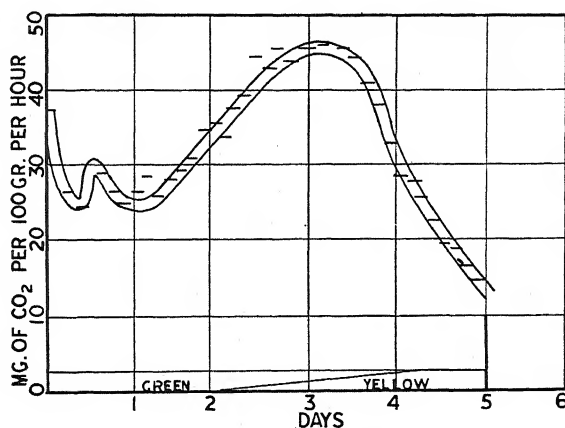


FIG. 2. Respiration record of detached wheat leaves during starvation. — — —, respiration record<sup>1</sup> (from exp. 1).

which is characteristic of the starvation respiration of the isolated leaves of many species. Respiration starts out with a high rate and declines from the beginning to considerably lower values. These give way without appreciable pause to rising values which reach a peak on the third day and from

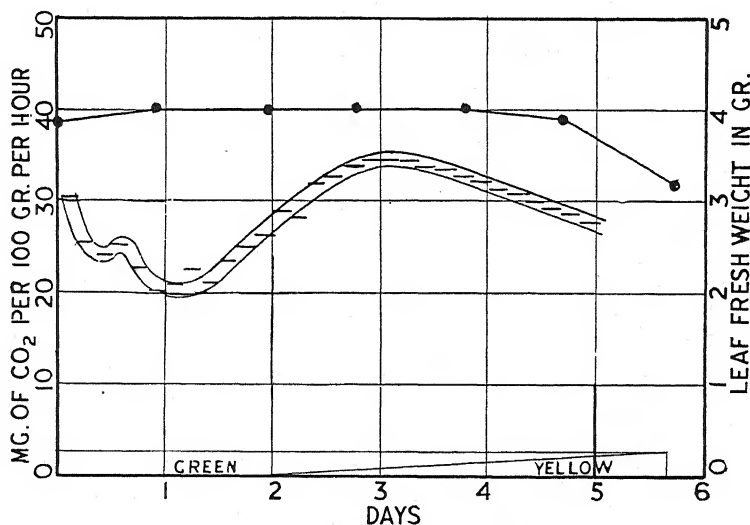


FIG. 3. Respiration record of detached wheat leaves during starvation. — — —, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 2).

<sup>1</sup> The author wishes to express his thanks to Dr. G. H. DUFF and Miss D. F. FORWARD for their kind permission to reproduce this respiration record, which was obtained by them in the course of their own work.

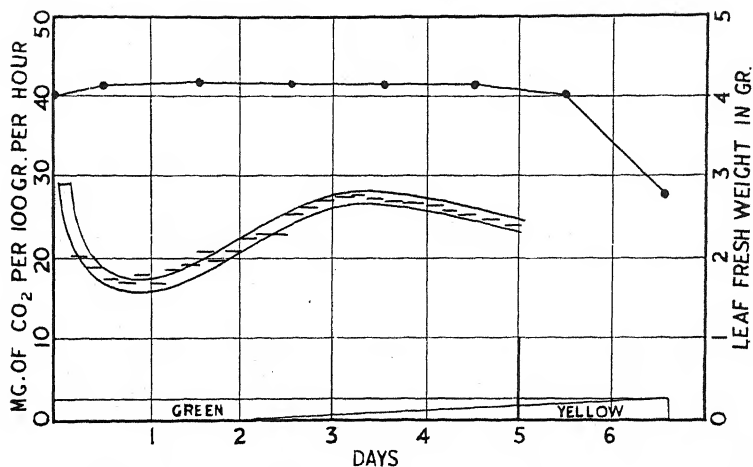


Fig. 4. Respiration record of detached wheat leaves during starvation. ———, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 3).

that point onward again decline. These variations in the rates of CO<sub>2</sub> emission are correlated in time with the visible changes taking place in the leaves. The green color first pales slightly, is then replaced by yellow, which finally gives way to darkening with exudation of sap into the intercellular spaces. A chart representing the time sequence of these changes is given with each respiration record.

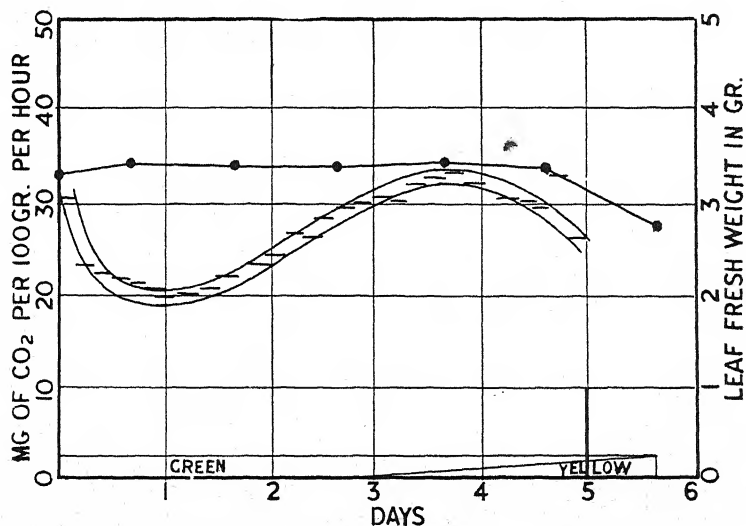


Fig. 5. Respiration record of detached wheat leaves during starvation. ———, respiration record; ●—●—●, changes in the leaf fresh weight (from exp. 4).

The final condition is obviously one of autolytic disorganization. Until it sets in, the fresh weight of leaves does not alter appreciably. As long as leaf weight remains constant it is clear that the permeability properties of the leaf cells have not been so altered as to impair their osmotic action and thus the fundamentals of cell organization are retained. With the appearance of darkening in color and exudation of sap, the leaf weight falls sharply. These are all coincident evidences of the onset of the final autolytic disorganization. At the same time  $\text{CO}_2$  emission becomes irregular in rate and thereafter can no longer be regarded as the product of an organized respiratory mechanism. The starvation respiration records are therefore considered to end at this point. Graphs showing fresh weight plotted against time are included with the respiration records in the three experiments in which fresh weight determinations were made. The slight initial rise which characterizes these graphs represents, no doubt, absorption of water to compensate the normal saturation deficit maintained in the attached leaf.

Though the four respiration records are obviously of the same general form, they nevertheless exhibit certain differences which require comment. In the first place, the characteristic transitions back and forth between a higher and lower level of drift are revealed in the record of experiment 1, but they are very poorly developed in the records of the materials grown in the greenhouse. These transitions are more fully described by DUFF and FORWARD (*loc. cit.*). Their significance is still somewhat problematical, but it may be noted here that the occurrence of a transition in the first declining phase of the respiration record promises to complicate the relation between sugar concentration and respiration rate during that phase in which it might be expected to be simplest. Before an R/S relation can be established for these materials, the significance of the transitions must be elucidated.

Other differences to be noted are those between the rates at which  $\text{CO}_2$  emission begins in the four records. Before a valid comparison of these initial rates can be made, they must be corrected for differences in the temperature at which the respiration experiments were performed. Using the Van't Hoff-Arrhenius equation the initial rates of experiments 2, 3, and 4 were corrected to the temperature of experiment 1, *i.e.*, to  $22.3^\circ \text{C}$ ., and the corrected values are brought together in table I.

From this table it is clear that the four initial respiration rates, though of the same order of magnitude, are not equal. The initial rates for experiments 2 and 4 are virtually the same, at 35.9 and 35.5 mg. of  $\text{CO}_2$  respectively, but the rate for experiment 3 is 34.0 and for experiment 1 is 37.7 mg. of  $\text{CO}_2$ . The maximum difference is therefore one of 3.7 mg. This is a significant difference and at first sight might be taken to indicate that the

leaves, at least in experiment 1, were not at a stage of the age sequence strictly comparable to that of the other experimental leaves. Experiments have shown (5) that with plants grown under the uniform artificial conditions maintained in experiment 1, several days are required to produce a change in the initial respiration rate of early mature leaves of 3.7 mg. of  $\text{CO}_2$ . The difference observed here between the initial rate of experiment 1 and that of experiment 3 is thus the equivalent of a maximum age difference of about three days under the uniform conditions. But since the leaves persist in the "mature" state for better than 20 days, it is clear that a maximum possible difference of 3 days over all is insufficient to call in question their comparability, so far as this can be determined from respiration rates alone.

TABLE I

COMPARISON OF INITIAL RESPIRATION RATES, MINIMAL TRANSITIONAL RESPIRATION RATES AT 22.3° C. AND TOTAL CARBON LOSS IN EXPERIMENTS 1 TO 4

	EXP. NO. 1	EXP. NO. 2	EXP. NO. 3	EXP. NO. 4
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Initial respiration rate in mg. $\text{CO}_2$ per 100 gm. of leaf fresh weight per hour .....	37.7	35.9	34.0	35.5
Transitional minimal rate in mg. $\text{CO}_2$ per 100 gm. of leaf fresh weight per hour .....	24.7	23.7	19.9	22.9
Total carbon loss in mg. $\text{CO}_2$ per 100 gm. of leaf fresh weight per hour .....	3941	3429	2797	3202

Table I also shows the minimal rates of  $\text{CO}_2$  emission to which the high initial rates decline. These have been similarly corrected to the temperature of 22.3° C. so that they are comparable with one another. The values for the minimal rates are evidently correlated with these for initial respiration and also with the total loss of carbon throughout starvation. If the minimal rates are assumed to measure the rate of the  $\text{CO}_2$  output of the leaf cells during a brief period in which their physiological state is distinct from preceding and succeeding states, it is evident that this state is characterized by  $\text{CO}_2$  output which rises and falls with the pitch of the whole respiration record. On the other hand it will be suggested later that on biochemical grounds the respiration record should be regarded as composite in character, falling into two more or less superimposed parts: (1) the decline from the high initial to low rate; (2) the hump. The correlations just indicated between the observed minimal rates and other measures are of the sort that would be expected were the minimal rates taken to represent transition values between the falling rates of the first declining phase and the rising

rates of the superimposed hump. A consideration of the influence of age upon the form of the respiration record and upon the magnitude of the minimal rates leads to the same conclusion. Where the first declining phase and the hump are well separated in time, as they are in juvenile and senile leaves, the minimal rates are low, and may or may not have a merely transitional significance. But where the two components of the record are considerably superimposed, as with leaves in the mature stage, the minimal rates are high and can hardly have any significance other than that of transitional values (5).

The figures for the total  $\text{CO}_2$  loss given in table I have not been corrected for the difference in temperature at which experiment 1, on one hand, and experiments 2, 3, and 4, on the other, have been carried out. Experiments by DUFF (6) revealed that within the limits of  $10^\circ$  to  $30^\circ$  C., temperature has no significant effect upon the total  $\text{CO}_2$  loss.

Changes in the sugar content of the leaves as starvation proceeds are shown for experiments 1 to 4 in figures 6 to 9, respectively. The most

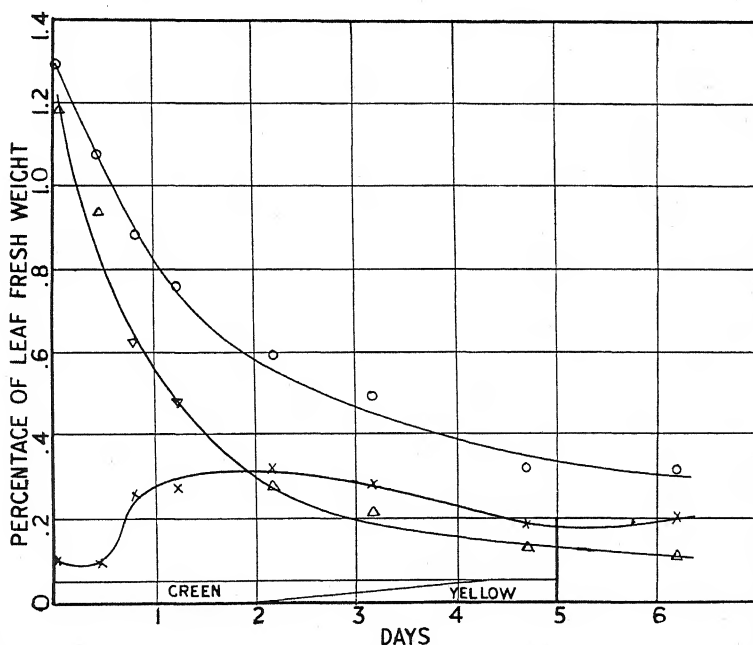


FIG. 6. Sugar content of detached wheat leaves during starvation.  $\times$ — $\times$ — $\times$ , reducing sugars;  $\Delta$ — $\Delta$ — $\Delta$ , invert sugars;  $\circ$ — $\circ$ — $\circ$ , total sugars (from exp. 1).

striking feature of the four graphs when compared is the variability of the initial sugar content. In this respect experiment 1 is unique since it starts out with a sugar reserve which is several times greater than the highest of

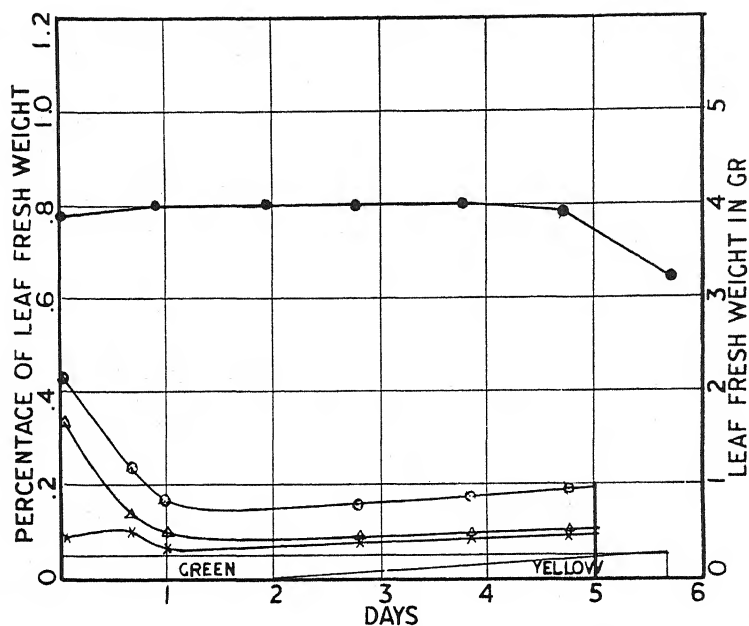


FIG. 7. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 2).

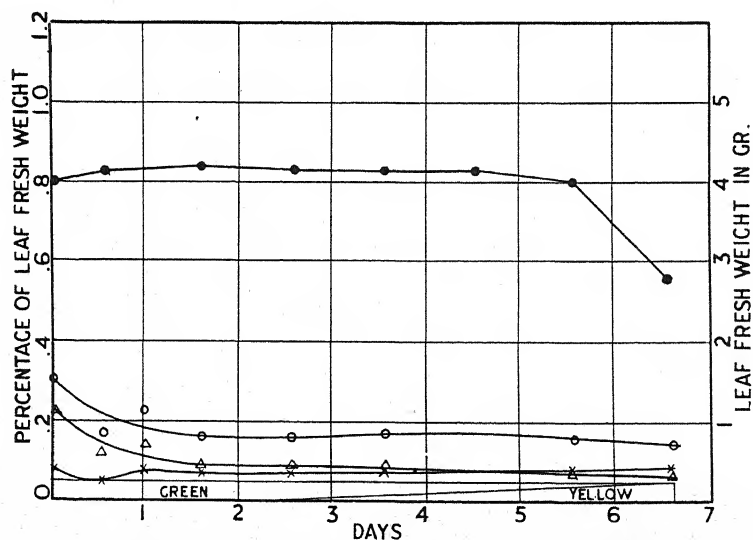


FIG. 8. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 3).

the others. These are the leaves that were grown under artificial conditions and were permitted 17 hours of continuous assimilation before they were isolated and put in darkness.

In all four experiments the reserve of sugars consists very largely of the non-reducing fraction referred to as "invert sugar," so that gross sugar loss is referable chiefly, if not entirely, to loss of invert sugar. Total sugar and invert sugar fall away in simple declining curves, most rapidly at first and more slowly later. This suggests that the rate of sugar loss might be proportional to the sugar content and this suggestion will be examined

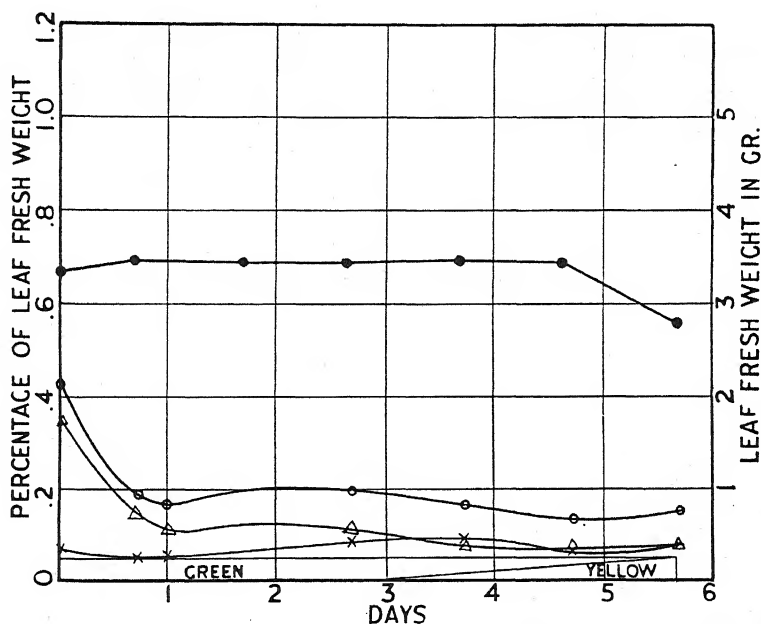


FIG. 9. Sugar content of detached wheat leaves during starvation. ●—●—●, changes in the leaf fresh weight; ×—×—×, reducing sugars; △—△—△, invert sugars; ○—○—○, total sugars (from exp. 4).

later in connection with the data from experiment 1. The reducing sugars show an unmistakable increase in the first half of the starvation period in experiment 1, which appears to indicate an excess of inversion over consumption during this period. In the experiments with leaves of low initial sugar content evidences of this increase in reducing sugars are perhaps to be seen but they are poorly developed and uncertain.

When the initial sugar content is low (experiments 2, 3, and 4) sugar loss proceeds in a manner such as to bring the sugar content to more or less steady values long before the end of the starvation sequence. This steady residual sugar value is very nearly the same in all of those experiments



(table II). It is also to be noted that the reducing and non-reducing fractions are present in similar proportions in each case. The residual sugar values are of some theoretical interest and will be considered at a later point of the paper.

Experiment 1 with leaves of high initial sugar content contrasts sharply with the others in respect to sugar depletion. It is obvious that sugar content is still declining at the end of the starvation, the final values are clearly of a higher order of magnitude than that of the residual sugars of experi-

TABLE II

CHANGES IN SUGAR DURING 5 DAYS OF STARVATION, IN MG. PER 100 GR. OF  
LEAF FRESH WEIGHT

	EXP. NO. 1	EXP. NO. 2	EXP. NO. 3	EXP. NO. 4
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Total sugars ..... { Initial .....	1291	432	302	424
..... { Residual .....	315	170	165	165
..... { Change in .....	- 976	-262	-137	-259
Invert sugars ..... { Initial .....	1187	345	222	360
..... { Residual .....	120	92	90	97
..... { Change in .....	-1067	-253	-132	-263
Simple sugars ... { Initial .....	104	87	80	64
..... { Residual .....	190	78	75	68
..... { Change in .....	+ 86	- 9	- 5	+ 4
Residual level is reached in approxi- mately .....	120 hr.	24 hr.	12-36 hr.*	24 hr.

\* In experiment 3, the graph showing variations in the sugar content of leaves within the first 48 hr. could be drawn in several ways, depending on the relative importance attributed to individual sugar values. As a consequence we can conclude that residual values are reached in this experiment sometime between 12 and 36 hours.

ments 2, 3, and 4, and the proportion in which the two fractions occur at the end is very different. The value for residual invert sugar in experiment 1 approaches that of the other experiments, but the reducing sugar fraction is very much higher (table II).

#### Analysis and interpretation of data

If the form of the respiration record and the totally different form of the graph for total sugar content of the starving leaves are compared it becomes immediately clear that the CO<sub>2</sub> output of the leaf cells cannot be wholly derived from the substances analyzing as sugars on every day of the starvation experience. In experiments 2, 3, and 4 in fact, inspection is sufficient to show that CO<sub>2</sub> production cannot be wholly derived from substances analyzing as sugars on any day. In these three experiments the proportion of the total CO<sub>2</sub> output which is contributed by the sugars is no

more than 11, 7, and 12 per cent. respectively. The leaves of experiment 1 had much the highest initial sugar content, and in them sugars contributed roughly 37 per cent. of the total  $\text{CO}_2$ . Taking this experiment as an example the  $\text{CO}_2$  output and the total sugar loss have been computed (in  $\text{CO}_2$  equivalents) for each of the 5 days of starvation and are given in table III.

TABLE III

CARBON DIOXIDE OUTPUT AND TOTAL LOSS OF SUGAR FOR 5 DAYS OF STARVATION

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
$\text{CO}_2$ output, mg. per 100 gm. leaf fr. wt. ....	689	726	1009	1008	509
Sugar lost in $\text{CO}_2$ equivalents, mg. per 100 gm. leaf fr. wt. ....	705	279	191	161	95
Sugar as percentage of daily $\text{CO}_2$ output .....	100%	38%	19%	16%	19%

This table leads to the conclusion that the  $\text{CO}_2$  produced on the first day is entirely derived from sugars, but subsequently the contribution of the sugars, both absolute and relative, becomes smaller. Hence it is obvious that some substance or substances not analyzed as sugars are contributing to the  $\text{CO}_2$  output in large and increasing proportions after the first day. These substances have not yet been identified and without any implication whatever as to their nature, or to the nature of their degradation products, they will be referred to hereafter as the "secondary substrate materials," in order to distinguish them from the substances analyzed as sugars and which form the "primary substrate materials." The difference between the daily  $\text{CO}_2$  production and the daily sugar loss expressed as  $\text{CO}_2$  equivalents gives, of course, the daily contribution of this secondary substrate to the  $\text{CO}_2$  emission. In experiments 2, 3, and 4 the secondary substrate contributes a substantial proportion of the daily  $\text{CO}_2$  even on the first day of starvation.

In figure 10 the respiration record of experiment 1 is reproduced and together with it are plotted the daily mean contributions of the primary and secondary substrates to the  $\text{CO}_2$  output. The configuration of these graphs strongly suggests that, as a first approximate formulation, the respiration record should be regarded as a composite one, owing the major peculiarities of its form to the form of its two components, which are:

1. The "primary substrate component," which starts out with high values and declines with time, representing the degradation of the original primary reserve of sugar.
2. The "secondary substrate component," which starts out with low values, rises to a maximum and then drops toward zero, representing the degradation of substances which have not yet been identified.

On this view the minimal values of the respiration record which occur toward the end of the first day must be regarded as transitional, being formed by the declining values of the primary and the rising values of the secondary substrate components. Their magnitude would, therefore, depend upon the form and timing of the two components and should not in these leaves be taken to have any more precise meaning than this.

If then the form of the respiration record is determined in this manner we must proceed to ask on what principles the forms of the two components themselves depend. In order to do this we may regard first the primary

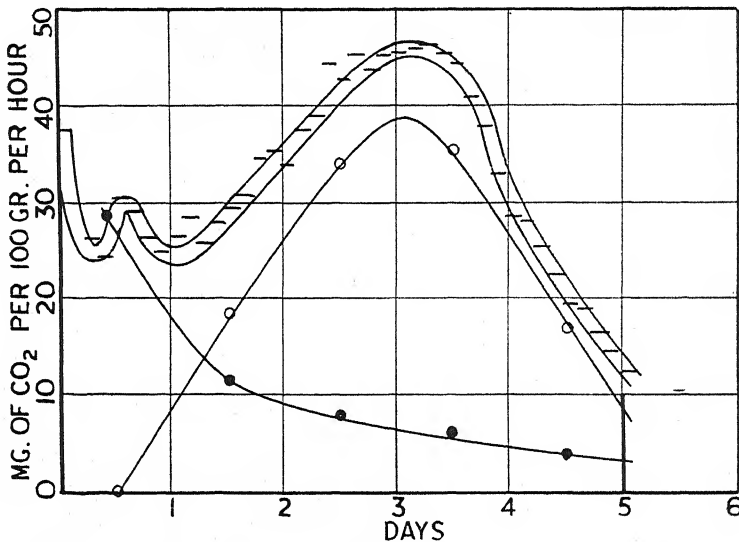


FIG. 10. Contributions of both substrates to  $\text{CO}_2$  production on various days of starvation.  $\text{---}$ , respiration record;  $\bullet\text{---}\bullet\text{---}\bullet$ , primary substrate contribution to  $\text{CO}_2$  output;  $\circ\text{---}\circ\text{---}\circ$ , secondary substrate contribution to  $\text{CO}_2$  output (from exp. 1).

component and to begin with, the gross consumption of the primary reserve of sugar over the whole starvation period.

The graphs for total sugar content (figures 7, 8, 9) in experiments 2, 3, and 4 show that the sugars fall off rapidly to residual values which are maintained with but slight evidence of drift or fluctuation (and that not uniform) from the second to the fifth day. In order to get properly weighted average values for the residual values, smooth curves were drawn through the determined points, as in figures 7, 8, and 9, but on a somewhat larger scale of sugar content relative to time. Ordinate values were then taken of points on these graphs separated by intervals of 4 hours throughout the whole period during which the residual values were maintained. The ordinate values were then averaged. The graphs show that the residual

values were much the same in all three experiments and the mean values which are recorded in table II are 170, 165, and 165 mg. for experiments 2, 3, and 4, respectively. The residual value therefore appears to be constant for comparable leaves. It has already been noted that the residual values consist of "invert" and "simple" fractions in roughly constant proportions. From this consideration of the residual values in experiments 2, 3, and 4, it is clear that the total consumption of sugars during the whole starvation period is in this case directly proportional to the initial sugar content.

Another point of interest lies in the fact that in experiments 2, 3, and 4 the residual values are reached long before autolytic disorganization destroys the respiratory system. This means that the final breakdown is not immediately dependent upon exhaustion of the primary reserve of sugar. We must look elsewhere, therefore, for the factor that precipitates the final stage of disorganization which is recognizable by coincident impairment of permeability, exudation, and irregular carbon dioxide output.

Some consideration must now be given to the chemical basis of the residual reducing value and its physiological significance. The residual values are reported as sugar by the analysis and in the discussion so far they have been tacitly assumed to be sugar in the same sense exactly as that larger fraction of the reported sugar which is consumed in the course of starvation. But, obviously, alternative views are possible and must be considered in the light of the effect that their possible confirmation would have upon the conclusions formulated from the present data.

Regarding the residual values first as sugar, four suggestions present themselves:

1. Starvation induces systematic changes, the effect of which is to prevent sugar utilization before the primary sugars are exhausted. This view cannot be maintained because it will be shown later that when the initial sugar content is high, the daily consumption of sugar throughout the whole starvation sequence is roughly proportional to the sugar content.
2. The unaltered respiratory system is subject to a threshold sugar content, an approximate measure of which is given by the residual sugar value. From the kinetic point of view such a state is improbable.
3. The residual values represent an equilibrium concentration of sugars in a system in which sugar is being liberated from combination and utilized in catabolism. The remarkable steadiness of the residual values during a long interval in which respiration rate is at first rising and then falling is difficult to reconcile with this view.
4. The residual values are to be attributed to sugar existing in the tissues in some combination which is broken down during the extraction process. Though no evidence of the reality of this suggestion can yet be put forward, it must be regarded as a serious possibility, if not a probability.

Finally we may note that the residual values might be attributed, partly if not wholly, to substances other than sugar. There is much evidence that such reducing substances occur in extracts of plant tissues (12, 13). A curious and constant feature of the residual reducing values, however, is that they are more than doubled after inversion under conditions held to be more or less selective for sucrose. This argues rather for the view that the residual substances are sugars than that they are not and indicates at least that they contain a considerable proportion of sugar.

Evidently the problem of the residual reducing values is obscure. Its solution will probably be found to involve several of the suggestions enumerated above. The fermentation technique, which might be expected to assist in its elucidation, gives anomalous results with extracts of wheat leaves, which indicate the presence of unidentified carbohydrate materials for the fermentation of which the potentialities of the micro-organic reagents are unknown. Much, therefore, must yet be done before a wholly satisfactory account of the residual reducing values can be given. In the meantime, conclusions must be limited to such as will remain valid however this matter turns out. One assumption will be necessary, however, and this is, that among the residual substances of comparable leaves the amount of non-sugar reducers is constant. From what is known of the residual reducing power before and after inversion this assumption appears to be reasonable. In this paper, therefore, the reducing values will continue to be treated altogether as sugar with the reservation that some constant quantity may have to be deducted from the *apparent* to give the *true* sugar content. Such a shift of the base line would entail subsequent numerical modifications, but the principles involved would remain unaffected.

So far the residual value of experiments 2, 3, and 4 alone have been considered and attention must now be directed to experiment 1, which seems to stand alone in this respect. Table II shows that the final low sugar value of experiment 1 is not reached early and maintained for many hours but is reached at the point of disorganization itself. This might mean that in the first experiment the final value is an "apparent residual value" only, and that it represents merely the value that the declining sugar content happens to have attained at the onset of breakdown, and not a value below which starvation will not depress the sugar content, *i.e.*, a value at which there is no apparent sugar consumption. The true residual value of experiment 1 on this view would be smaller than the final value actually achieved. Were the leaves of experiment 1 physiologically comparable to those of the other experiments, we might expect their residual values to be equal. At a later point of the paper an estimate of the true residual value, *i.e.*, the sugar content corresponding to zero sugar loss for experiment 1, will be made. In the meantime it would seem that the apparent gross consumption of reserve

sugar in isolated starving leaves of low to moderate initial sugar content is equal to the initial sugar minus a residual value which is constant for comparable leaves. But at least in leaves of high initial sugar content the apparent consumption may be smaller than that given by this simple rule and there may be a sugar surplus at the time of breakdown.

The form of the primary component of the respiration record also involves a consideration of the question of the change in rate of sugar consumption as starvation proceeds. For this purpose we are confined to the data of experiment 1, since in the other experiments the sugars were depleted too soon to yield a series of successive daily sugar losses.

TABLE IV

DAILY SUGAR CONSUMPTION AND DAILY INITIAL SUGAR CONTENT (EXPERIMENT 1)  
(MG. OF SUGARS PER 100 GR. LEAF FRESH WEIGHT)

	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Sugar initially present .....	1291	810	620	490	380
Sugar consumed (observed)	481	190	130	110	65
Sugar consumed (calculated)	459	237	150	91	40

The daily consumption of sugar and the daily initial sugar content for the 5 days of experiment 1 are given in table IV and plotted against each other in figure 11. The distribution of points suggests a linear relation

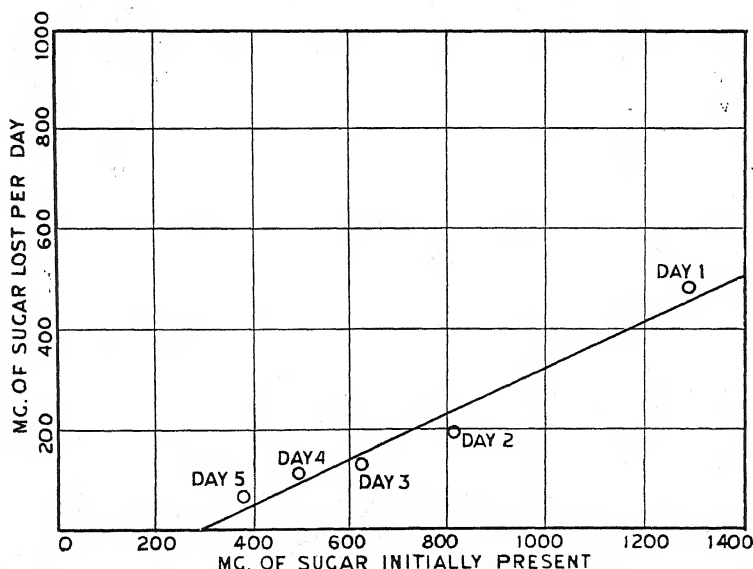


FIG. 11. Daily mean rate of sugar loss and daily initial sugar content (from exp. 1).

within this range of values, and accordingly the constants of the equation of the straight line of best fit have been calculated by the method of least mean squares. The equation is  $y = 0.46x - 135$ . By use of this equation values for daily sugar consumption have been calculated and in table IV they are compared with those observed. Considering the nature of the primary data, the fit, though far from precise, seems close enough to warrant the conclusion that the relation is rectilinear.

If so, this relation may be formulated in simple terms for experiment 1: 100 gm. of leaves consume each day 135 mg. less than 46 per cent. of the initial sugar content of that day until breakdown occurs. More generally: the daily mean rate of sugar consumption is proportional to the sugar content. This is the principle upon which the form of the primary component of the respiration record is determined. We conclude, therefore, that the first declining phase of the respiration record takes its character from the metabolism of the original primary reserve of sugar, the changing rates of which are a simple function of its diminishing concentration.

This formulation of the data makes it possible to estimate the true residual value for experiment 1. This is obviously given by the intercept of the line  $y = 0.46x - 135$  on the axis of the abscissae, since in the other experiments the residual value corresponds to the value of  $x$  when  $y = 0$ . The line intercepts the axis at 293 mg., which is therefore the residual reducing value for experiment 1. This deduced value is so close to the final sugar value reported by analysis (i.e., 315 mg.) that one would hardly be justified in distinguishing between them. It appears, therefore, that the leaves of experiment 1 with their high initial sugar content, became finally disorganized as they approached a point of sugar depletion corresponding to that which the leaves of experiments 2, 3, and 4 entered upon on the second day of the starvation sequence. Evidently two physiological states are represented here. Furthermore, the residual value for experiment 1 is manifestly higher than the corresponding values for the other experiments. This suggests that criteria of comparison in respect to sugar metabolism might possibly be developed from the constants relating daily mean rate of sugar loss to sugar content.

Owing to the low initial sugar content of the leaves in three of the experiments it is not possible to do more here than attempt a merely preliminary examination of this suggestion. For this purpose the available data are assembled in figure 12. We have 5 points for experiment 1, and 2 points for experiments 2 and 4. Data for experiment 2 have not been used for this comparison since, in this experiment as explained earlier in the paper, no definite conclusion could be drawn as to the time when the residual sugar value was reached. The derivation of the line for experiment 1 has been given; that for experiments 2 and 4 seems fairly well

supported by the fact that the corresponding points for the two independent experiments are practically coincident.

This graphic presentation has the effect of comparing the experimental materials on a two-fold basis, the criteria being the magnitude of the residual reducing value (intercept on the axis of  $x$ ) and the coefficient of proportionality between daily mean rate of sugar loss and sugar content (slope of the line). Evidently experiments 2 and 4 are strictly comparable in both respects and are, in fact, practically identical. These materials were grown in the greenhouse in bright weather; they had low to medium initial sugar content; their low residual values and high slope constant are

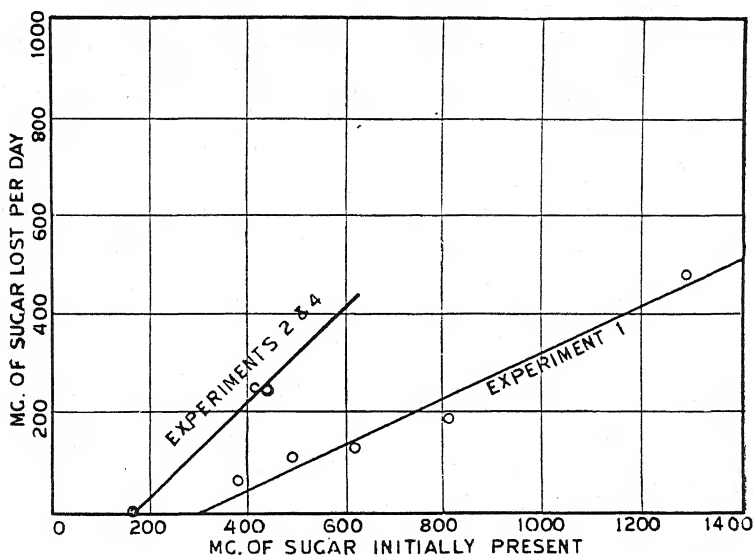


FIG. 12. Relation between sugar content and daily mean rate of sugar loss.

held in common. In experiment 1, the materials for which were grown under steady artificial conditions, we have by far the highest sugar, and a high residual value, but a low coefficient of proportionality. Experiment 1, therefore, stands apart from the others in respect to both criteria.

Before criteria such as these can be properly employed, their physiological significance must be clear. The significance of the residual reducing value has already been discussed. It is not yet clear and may have a purely chemical significance. Should it turn out that the whole of the residual reducing power must be attributed to extraneous interfering substances or the sum of such substances and combined sugar, then all the lines would pass through the origin and this criterion would disappear. Under all other circumstances some real residual sugar must always remain for consideration. The significance of the coefficient of proportionality, on the



other hand, would be a purely physiological one. It would represent the activity of any given sample of leaves at a given temperature in the metabolism of sugar under starvation conditions. Thus two activities appear to be represented in the three experiments. No measurable correlative of activity can as yet be suggested.

We may now turn to the secondary substrate component of the respiration record and review the sequence in time of the changes in its catabolism. As a necessary consequence of the form of the respiration record, the catabolism of the secondary substrate must begin with low daily rates, rising to a maximum roughly coincident with that of the respiration record, then falling toward zero. Without knowledge of the identity of the secondary substrate materials, the analysis cannot be pressed too far, but it seems certain that the liberation of the secondary substrate quota of the  $\text{CO}_2$  output involves the degradation of a variety of substances.

The disappearance of chlorophyll at rates corresponding roughly to the rise and fall of the respiration record is easily noted visually. GODWIN and BISHOP (7) describe the degradation of the cyanophoric glucoside of the cherry laurel leaf during starvation and find that the changes in the rate of its disappearance are roughly coincident with those of the hump phases of the respiration record. Finally, the work of DELEANO and of YEMM suggests that protein degradation, if examined in relation to the respiration record, might show a similar time relation.

These biochemical changes have their functional correlatives and BLACKMAN (2) has described the inactivation, first of the photosynthetic, and finally of the respiratory system in the starving cherry laurel leaf. Clearly, the degradations which accompany the liberation of the secondary substrate quota of the  $\text{CO}_2$  output are such as to affect protoplasmic organization. There is little evidence on the extent to which these degradations are coincident or successive, but since the physiological effects are undoubtedly successive and orderly, it is probable that there is a fairly definite order of biochemical events. This unknown order of events determines the manner in which the secondary substrate quota of the  $\text{CO}_2$  output is liberated and thus determines the form of the final phases of the respiration record.

### Summary

Data are presented on the changes in the rate of  $\text{CO}_2$  emission and in the content of simple and complex sugars which proceed in the cells of isolated, first foliage leaves of wheat starving in the dark. The dual form of the highly characteristic changes in the rate of  $\text{CO}_2$  emission is shown to have its origin in the degradation of two categories of substances to yield respiratory substrate. The two categories are the original primary reserve of

sugars on the one hand, and on the other, a variety of substances, some of which are of significance in protoplasmic organization. The manner of depletion of reserve sugar controls the course of starvation metabolism in its early stages. The depletion of secondary substrate materials guides the metabolic drift in the later stages and finally causes it to terminate suddenly in autolytic disorganization.

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# DISTRIBUTION OF DIFFERENT NITROGEN FRACTIONS, SUGARS AND OTHER SUBSTANCES IN VARIOUS SECTIONS OF THE PINEAPPLE PLANT GROWN IN SOIL CULTURES AND RECEIVING EITHER AMMONIUM OR NITRATE SALTS\*

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(WITH ELEVEN FIGURES)

## Introduction

Data bearing on the behavior of pineapple plants grown in water cultures and receiving either ammonium or nitrate salts as sources of nitrogen were presented in a former paper (6). This present report is concerned with similar information for plants grown in soil- instead of water-cultures. One may be justified in stating that any differences in the behavior between plants of the water- and soil-culture series with comparable nitrogen nutrition are due to chemical changes in the nitrogen of the soil by bacteria. The oxidation of ammonium to nitrate ions in the soil by bacteria, which has played a very important rôle in the present studies, is a well-known fact and needs no discussion.

The literature of plant nutrition with either ammonium or nitrate salts has been reviewed in the former two papers of this series (5, 6). For a more thorough and extensive review the papers by NIGHTINGALE (4) and McKEE (2, 3) are recommended.

## Methods

The plants were grown in two contiguous soil plots, the one designated, for convenience, the ammonium series and the other the nitrate series. Be-

TABLE I  
AMMONIUM NUTRIENT SOLUTION

GRAMS	NAME OF SALT	GRAMS PER LITER				
		N	P	K	S	Fe
300	Ammonium sulphate .....	69.5			72.0	
50	Di-ammonium phosphate ..	1.1	1.2			
20	Potassium sulphate .....			9.1	3.6	
25	Iron sulphate .....				2.8	5.0
Total .....		70.6	1.2	9.1	78.4	5.0
Gm. per plant per treatment .....		3.5	0.06	0.45	4.9	0.25
Gm. per plant per cycle (12 mo.) .....		28.0	0.48	3.60	39.0	2.00

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ginning when the plants were four months old, *i.e.*, on April 13, 1934, those in each plot were treated, once a month for the subsequent 8 months, with 50 ml. of either an ammonium or a nitrate solution of the compositions as shown in tables I and II.

TABLE II  
NITRATE NUTRIENT SOLUTION

GRAMS	NAME OF SALT	GRAMS PER LITER					
		N	P	K	S	Na	Fe
370	Sodium nitrate .....	61.00				81.00	
6.7	Di-potassium phosphate .....		1.2	3.1			
15	Potassium sulphate .....			6.8	2.7		
25	Iron sulphate .....				3.0		5.0
Total .....		61.00	1.2	9.9	5.7	81.00	5.0
Gm. per plant per treatment .....		3.05	0.06	0.49	0.29	4.00	0.25
Gm. per plant per cycle (12 mo.) .....		25.00	0.48	3.90	2.30	32.00	2.00

The solution was applied in the axils of mature leaves which, depending on the age of the plants, belonged either to group B or C; that is, to old or mature leaves. At the end of about eleven months, ten plants from each lot were harvested. Their leaves were separated into groups and these, with the stem, into sections according to the plan mentioned in a preceding publication (6). A brief description of the sampling and analytical techniques is presented.

The leaves were detached from the stem and placed in groups according to age. The leaves on the basal portion of the stem, having developed earlier than those on the medial and apical portions, were placed in separate groups. Three such groups of leaves were formed: the C-group composed of thoroughly mature leaves; the D-group of active and fully expanded leaves, and the E-group of young leaves only. The stem was also subdivided into sections corresponding to the respective leaf groups attached thereto, and were designated accordingly. Stem sections D and E were combined to furnish sufficient material for analysis. Leaves of the different groups were subdivided into five sections as follows: (1) basal, non-chlorophyllous, in young leaves mostly of meristematic tissues; (2) transitional, subchlorophyllous; (3) lower chlorophyllous; (4) intermediate chlorophyllous; (5) terminal chlorophyllous. The basal section comprised, in all leaf groups, the youngest, and the terminal the oldest tissues of the leaf.

The analytical procedure already discussed (6) was adopted, with the exception that fractions of soluble mono-amino nitrogen and basic nitrogen as designated in the former publication were not analyzed separately in these studies but were combined. The nitrogen of both fractions is designated either as rest soluble nitrogen or as combined amino and basic nitrogen.

### Experimentation

The various data are presented in tables IV to VIII and in figures 1-A to 9-A and 1-B to 9-B. They are concerned with the weight values of the plants and their various sections, the distribution of moisture, electrical resistance (ohms), titratable acidity and pH, assimilated and non-assimilated nitrogen, total and reducing sugars, and sucrose in the plant tissues.

### PLANT WEIGHTS

The weight of the individual plants of both series used for analysis varied considerably. The plants with ammonium nutrition ranged in weight from 3200 to 4500 gm. with a mean value of 3800 gm. while those of the nitrate series ranged from 2400 to 3600 gm. with a mean of 3000 gm. It has not been definitely established whether the difference in mean weight of 800 gm. between the plants of the two series was caused by differences in their nitrogen nutrition or to other causes.

In the protocol below (table III) is reported the mean weight values of the different leaf and stem sections of the two series of plants. The last column shows the ratio values of section weights of the ammonium plants to section weights of the nitrate plants.

TABLE III

MEAN WEIGHT VALUES OF STEM AND LEAF SECTIONS IN AMMONIUM AND NITRATE SERIES

PLANT SECTIONS	AMMONIUM PLANTS		NITRATE PLANTS		RATIO AMMONIUM/NITRATE PLANTS
	SECTIONS	GROUP	SECTIONS	GROUP	
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	
Mature leaves C					
Base (white)	1 68		58		
	2 170		188		
	3 257		168		
	4 338		199		
Tip	5 272	1105	147	760	1.45
Active leaves D					
Base (white)	1 145		145		
	2 240		260		
	3 327		203		
	4 390		247		
Tip	5 302	1404	187	1042	1.35
Young leaves E					
Base (white)	1 86		88		
	2 153		153		
	3 126		109		
Tip	4 70	435	65	415	1.05
Stem					
A and B (basal)	229		194		
C to E (apical)	121	350	111	305	1.15

## MOISTURE CONTENT OF SECTIONS

The water content of the different sections of the leaves and stem of the plants of both series was determined for the purpose of comparing the relative succulence of the two lots of plants. Table IV and figures 1-A and 1-B show that the moisture content of the ammonium series was, with a few minor

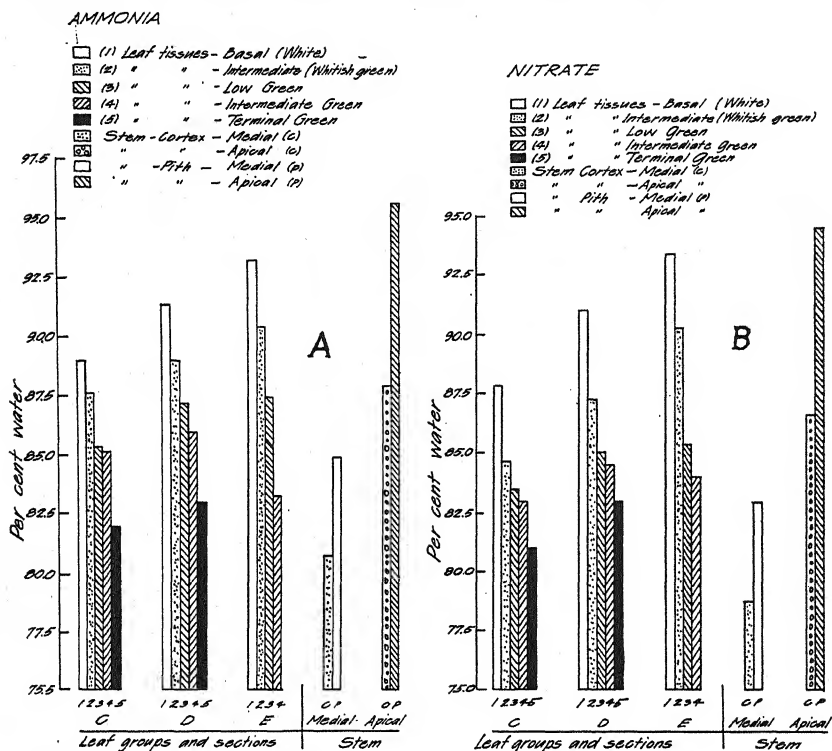


FIG. 1-A. Percentage water in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, in (C) the cortex and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 1-B. Percentage water in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and in (C) the cortex and (P) pith of the medial and apical sections of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

exceptions, greater than that of the nitrate group. The difference in the moisture content of comparable sections is, however, neither very great nor as significant as that found previously when the plants were grown in water cultures. The reason for this condition may be the modified nitrogen nutri-

tion of the plants in soil cultures as the result of oxidation of ammonium ions to nitrate in the soil. The findings are nevertheless in harmony with those obtained from plants grown in the nutrient solution cultures (6).

### ELECTROLYTES

This is a collective term and comprises all substances of the extracted sap which, when placed between two electrodes in aqueous solution, conduct electric current. The presence of non-electrolytes, such as sugars, etc., interferes to some extent with the free movement of electrolytes and may increase the electrical resistance of the solution.

TABLE IV

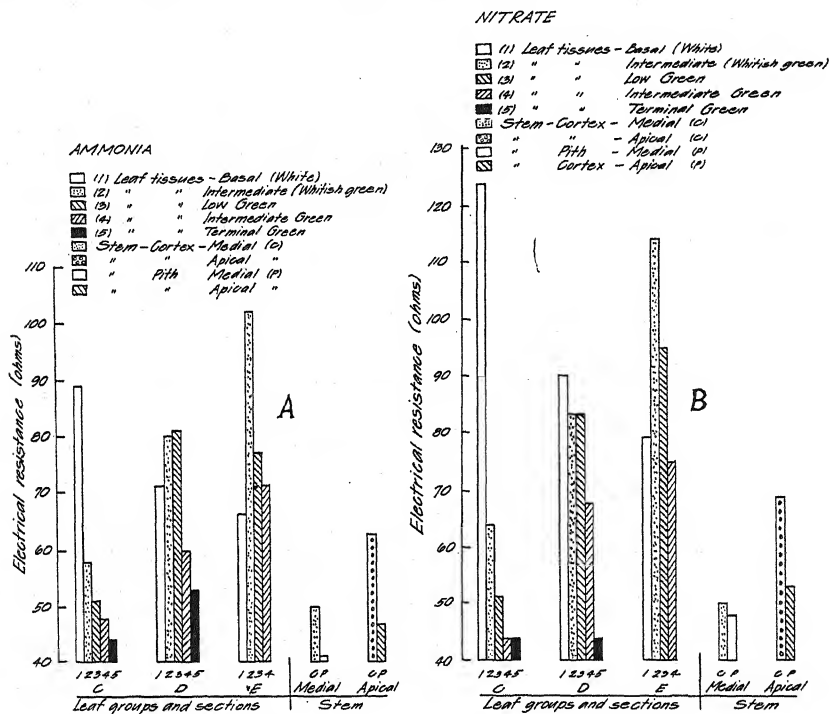
DISTRIBUTION OF MOISTURE, ELECTRICAL RESISTANCE, TITRATABLE AND FREE ACIDITY OF DIFFERENT SECTIONS OF THE LEAVES AND STEM OF PINEAPPLE PLANTS GROWN IN THE SOIL AND RECEIVING EITHER AMMONIUM OR NITRATE NITROGEN

PLANT SECTIONS	NUTRITION SERIES							
	AMMONIUM				NITRATE			
	WATER	RESISTANCE	ACIDITY		WATER	RESISTANCE	ACIDITY	
			CITRIC ACID	PH			CITRIC ACID	PH
Leaves	%	ohms	%		%	ohms	%	
Mature								
C1 (Base)	89.0	89	0.03	5.0	87.8	124	0.03	5.0
C2 .....	87.7	58	0.03	5.0	84.6	64	0.19	4.6
C3 .....	85.3	51	0.45	4.6	83.0	51	0.96	4.4
C4 .....	85.2	48	0.57	4.5	83.0	44	1.82	4.0
C5 (Tip)...	82.0	44	1.75	3.8	81.4	44	2.24	3.8
Active								
D1 (Base)	91.4	71	0.05	5.2	91.0	90	0.10	5.0
D2 .....	89.0	80	0.03	5.0	.....	83	0.22	4.6
D3 .....	87.2	81	0.48	4.2	85.0	83	0.70	4.2
D4 .....	86.0	60	1.40	3.8	84.5	68	1.60	3.7
D5 (Tip)...	83.0	53	1.45	3.6	83.0	44	2.24	3.5
Young								
E1 (Base)	93.2	66	0.05	5.2	93.4	79	0.13	5.2
E2 .....	90.3	102	0.07	5.0	90.2	114	0.16	5.0
E3 .....	87.4	77	1.00	3.7	85.4	95	0.64	4.0
E4 (Tip)...	83.2	71	1.35	3.8	84.0	75	1.40	3.6
Stem								
Medial								
Cortex .....	80.7	50	0.13	5.0	.....	50	0.10	5.0
Pith .....	84.8	41	0.16	5.0	.....	48	0.16	5.0
Apical								
Cortex .....	87.8	63	0.23	5.2	.....	69	0.19	5.0
Pith .....	95.6	47	0.26	5.0	.....	53	0.26	5.0

The data as presented in table IV and figures 2-A and 2-B show that there is a trend of electrolyte accumulation in the mature terminal sections of the leaves, while the young white basal and intermediate sections contain corre-



spondingly smaller quantities. It will be observed that the sections of the youngest leaves, *i.e.*, E leaves of both plants supplied with ammonium and with nitrate, contain considerably smaller amounts of electrolytes. This condition occurring more frequently in the youngest leaves of the plant, may be associated with certain physiological changes in the tissues undergoing rapid differentiation. Evidence obtained elsewhere on the distribution of



certain forms of soluble organic nitrogen and carbohydrates in the various sections of the E group of young leaves is in harmony with this hypothesis. The electrolytes ordinarily present in the extracted plant sap are potassium, nitrate, amino acids, and various hydroxy-acids. Calcium and magnesium

salts, amides, etc., occur in comparatively smaller amounts than the above mentioned substances. Detailed accounts on the distribution of different electrolytes in different parts of the plant will be presented in a forthcoming publication.

The data show, in general, that electrolytes are present in greater amounts in the older than in the younger groups of leaves and in greater amounts in the older tissues of any one group of leaves.

### ACIDITY

Titrateable acidity and pH were determined on the extracted sap and are presented in table IV. The former values are expressed as per cent. citric acid, although other organic acids besides citric normally occur in the tissues.

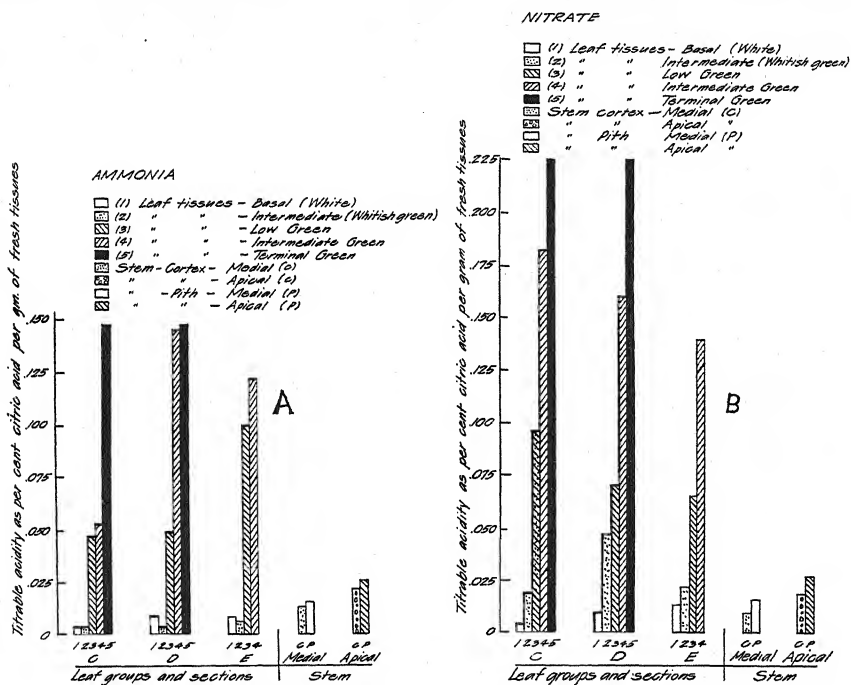
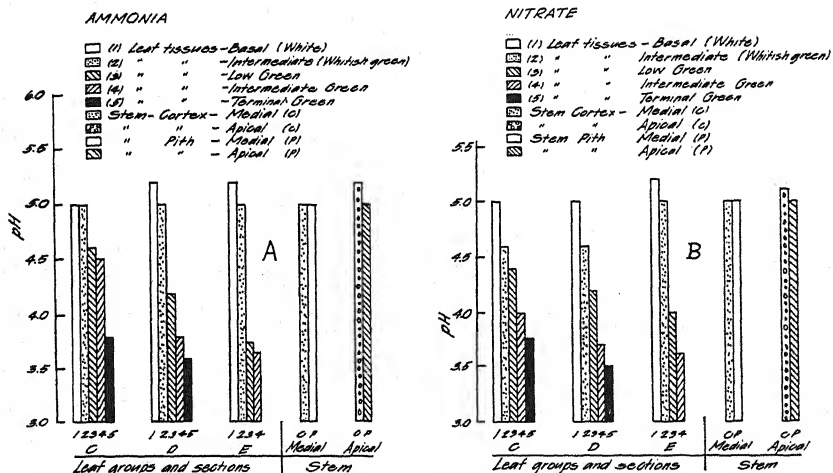


FIG. 3-A. Titratable acidity as per cent. citric acid of the extracted sap (1) of the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 3-B. Titratable acidity as percentage of citric acid of the extracted sap of (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature, (D) active, and (E) young leaves, and of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

The pH values were obtained colorimetrically, after dilution of the sap with water and proper masking of inherent colors and turbidity. Figures 3-A and 4-A present the distribution of titratable and free acidity (pH) in the various sections of the leaves and stem of the plants receiving ammonium nutrition; figures 3-B and 4-B present similar data for corresponding sections of the plants supplied with nitrate.

There are no appreciable differences in the pH values of corresponding plant sections of the two different lots of plants. Those that may be observed are in most cases within the range of experimental errors. The amounts of total acids were greater for the nitrate than for the ammonium lot of plants. The differences are comparatively great and significant. The causes for the difference in the acidity of corresponding sections of the two different lots of plants cannot be explained without additional experimental evidence. CLARK (1) working with tomato plants grown respectively in ammonium and nitrate solution cultures obtained similar results.



#### DISTRIBUTION OF NITROGENOUS FRACTIONS

The total nitrogen content of the different sections of the leaves and stem of both lots of plants was divided into non-assimilated, or inorganic nitrogen, and into assimilated, or organic nitrogen. The former consisted mostly of

TABLE V

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, AND INSOLUBLE ORGANIC NITROGEN FRACTIONS PER GRAM OF FRESH WEIGHT IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF *Amanus comosus* (L.) MERR. GROWN IN TWO DIFFERENT SOIL PLOTS AND RECEIVING MONTHLY APPLICATIONS OF EITHER AMMONIUM OF NITRATE NITROGEN

PLANT SECTIONS	NUTRITION SERIES																		
	AMMONIUM-NITROGEN									NITRATE-NITROGEN									
	ORGANIC									INORGANIC									
	INORGANIC			ORGANIC						INORGANIC			ORGANIC						
				SOLUBLE			INSOLUBLE						SOLUBLE			INSOLUBLE			
NH <sub>4</sub>	NO <sub>3</sub>		GLUTA-MINE	ASPAR-AGINE	REST	AMIDE	MONO-AMINO	BASIC	HUMIN	NH <sub>4</sub>	NO <sub>3</sub>		GLUTA-MINE	ASPAR-AGINE	REST	AMIDE	MONO-AMINO	BASIC	HUM
mg.	mg.		mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.		mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves																			
Mature																			
C1 (Base) .....	0.014	0.488		0.075	0.089	0.504	0.062	0.358	0.084	0.067	0.043	0.070	0.734	0.088	.....	0.084	0.084	0.0	0.0
C2 .....	0.010	0.506		0.070	0.046	0.514	0.062	0.297	0.112	0.084	0.047	0.069	0.734	0.133	.....	0.193	0.193	0.1	0.1
C3 .....	0.009	0.178		0.088	0.072	0.480	0.081	0.264	0.220	0.178	0.048	0.082	0.612	0.231	.....	0.480	0.480	0.2	0.2
C4 .....	0.010	0.076		0.082	0.101	0.642	0.136	1.080	0.480	0.250	0.048	0.075	0.528	0.215	.....	0.660	0.660	0.2	0.2
C5 (Tip) .....	0.009	0.068		0.081	0.128	0.514	0.154	1.080	0.424	0.202	0.041	0.082	0.442	0.231	.....	0.575	0.575	0.2	0.2
Active																			
D1 (Base) .....	0.013	0.460		0.090	0.150	0.750	0.067	0.364	0.113	0.055	0.038	0.115	0.824	0.098	0.350	0.119	0.119	0.0	0.0
D2 .....	0.008	0.383		0.054	0.059	0.468	0.059	0.258	0.103	0.073	0.050	0.058	0.493	-0.090	0.310	0.200	0.200	0.0	0.0
D3 .....	0.011	0.153		0.064	0.039	0.382	0.086	0.432	0.168	0.120	0.032	0.070	0.403	0.158	0.414	0.416	0.416	0.1	0.1
D4 .....	0.012	0.058		0.071	0.075	0.628	0.120	0.828	0.336	0.230	0.036	0.055	0.259	0.160	0.932	0.460	0.460	0.2	0.2
D5 (Tip) .....	0.010	0.058		0.076	0.086	0.605	0.154	1.230	0.316	0.260	0.038	0.065	0.259	0.130	0.930	0.500	0.500	0.2	0.2
Young																			
E1 (Base) .....	0.012	0.224		0.084	0.104	0.650	0.080	0.750	0.154	0.080	0.040	0.120	0.768	0.100	0.530	0.166	0.166	0.0	0.0
E2 .....	0.009	0.175		0.062	0.062	0.403	0.070	0.415	0.096	0.084	0.032	0.054	0.365	0.073	0.490	0.132	0.132	0.0	0.0
E3 .....	0.009	0.070		0.061	0.058	0.258	0.092	0.684	0.185	0.141	0.043	0.081	0.235	0.113	0.790	0.292	0.292	0.1	0.1
E4 (Tip) .....	0.010	0.029		0.071	0.069	0.275	0.133	1.010	0.264	0.214	0.017	0.086	0.235	0.148	0.890	0.440	0.440	0.1	0.1
Stem																			
Medial																			
Cortex B .....	0.043	0.383		0.099	0.159	1.230	0.129	0.448	0.176	0.157	0.055	0.185	1.430	0.101	0.592	0.196	0.196	0.1	0.1
Pith B .....	0.052	0.440		0.091	0.190	1.560	0.095	0.414	0.170	0.107	0.055	0.226	1.720	0.095	0.560	0.198	0.198	0.1	0.1
Apical																			
Cortex C .....	0.036	0.266		0.128	0.200	1.170	0.105	0.623	0.126	0.100	0.048	0.290	1.075	0.115	0.666	0.246	0.246	0.1	0.1
Pith C .....	0.045	0.355		0.168	0.328	1.500	0.116	0.584	0.168	0.098	0.087	0.376	1.290	0.125	0.643	0.180	0.180	0.0	0.0

nitrate nitrogen which was absorbed by the roots directly from the soil, and of ammonium nitrogen which may represent either non-assimilated nitrogen or nitrogen derived from the proteins of senile tissues by enzymatic hydrolysis, or that resulting through reduction of nitrate. The organic or assimilated nitrogen was divided into soluble and insoluble fractions. The soluble fraction of the organic nitrogen was segregated into glutamine, asparagine, and rest soluble (amino plus basic) nitrogen. The insoluble portion was fractionated, after hydrolysis with 20 per cent. sulphuric acid, into amide, amino and basic nitrogen, and humin nitrogen.

The distribution of these nitrogeaneous fractions in the different sections of the leaves and stem may be seen in tables V to VII and in figures 5 to 8.

The data presented below comprise values obtained for leaf and stem tissues but not for roots. The great difficulty encountered in extricating roots from the soil has prevented us from obtaining reliable samples for these studies.

#### STEM

Only the medial and apical sections of the stem, to which leaf groups C, D, and E were attached, were reserved for chemical analysis. The basal section was discarded on account of the senile condition of the tissues. The cortex was separated, as carefully as possible, from the pith before analysis. The results obtained with the two different lots of plants were as follows:

**AMMONIUM SERIES.**—The analytical data presented in figure 5-A show that the amounts of ammonium nitrogen in the various stem sections were very small as compared with those of nitrate nitrogen, in spite of the fact that the plants were supplied exclusively with ammonium salts. The amounts of both ammonium and nitrate were greater in the pith than in the cortex, possibly because of the preponderance of water-conducting elements in the former tissue. The small amounts of ammonium can be explained either by the assumption of its conversion to nitrate in the soil before absorption, or by its rapid assimilation, as was found in our former studies (6). It is possible that both processes operated simultaneously.

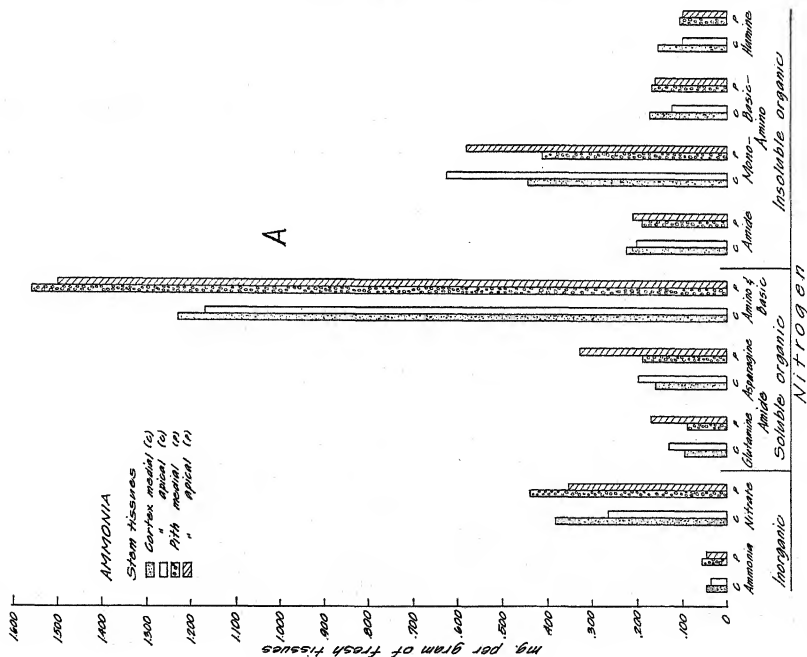
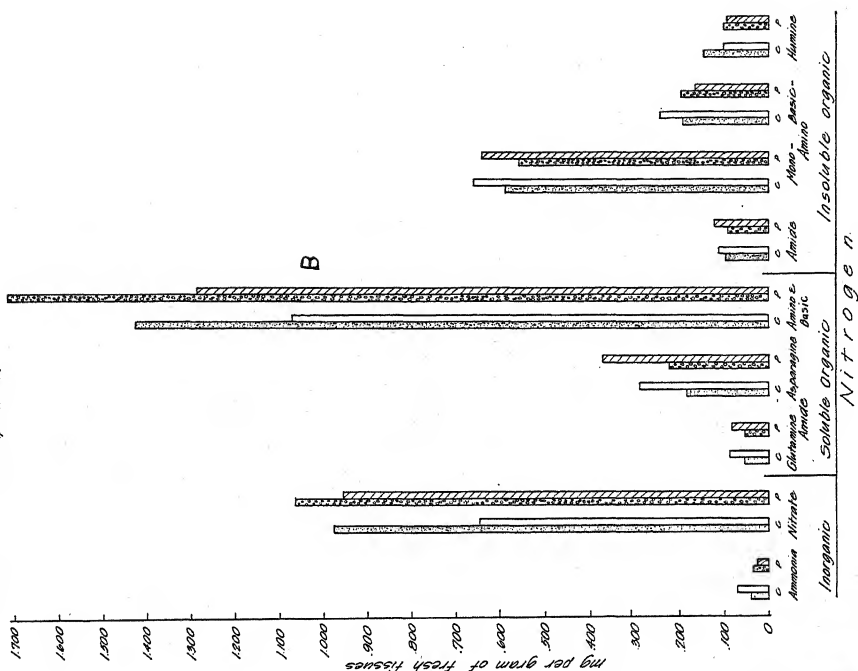
The values for the various fractions of soluble organic nitrogen were comparatively high throughout the plant. The combined values of amino and basic nitrogen especially appear very high when they are contrasted with the amide fractions (glutamine plus asparagine). The values of all such fractions, with a single minor exception, were consistently greater for the pith than for the cortex. The values for the different fractions of insoluble

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FIG. 5-A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in the cortex and pith of the medial and apical sections of the stems of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 5-B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in the cortex and pith of the medial and apical sections of the stems of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

NITRATE  
Stem tissues  
Cortex tissues - Apical (a)  
" " " " " (a)  
Pith tissues - Apical (a)  
" " " " " (a)



organic, or protein nitrogen, however, were usually greater for the cortex than for the pith. The greater amounts of protein and smaller amounts of soluble organic nitrogen present in the cortex indicate a greater rate of conversion of the soluble organic nitrogen to protein in the cortex than the pith. The formation of greater amounts of protein in the cortex were favored by the presence of relatively larger quantities of sugars.

**NITRATE SERIES.**—The data in figure 5-B and table V show that the amounts of ammonium in the different sections of the stem were very small and these amounts may represent nitrogen which had been derived from proteins by enzymatic hydrolysis or through nitrate reduction. The amounts of nitrate, however, were very great and represent nitrogen in its native state, *i.e.*, as it was absorbed by the roots from the soil and before its conversion to one of its many organic forms. The amounts of nitrate were greater in the pith, possibly for the same reasons already mentioned. The soluble organic nitrogen content, particularly the combined fractions of amino and basic nitrogen, was very high. The amounts of asparagine were slightly greater in the plants supplied with nitrate than in those supplied with ammonium, whereas the opposite condition was true with glutamine.

Protein was present in moderate amounts, the values comparing favorably with those of the preceding series.

#### MATURE LEAVES (C-GROUP)

**AMMONIUM SERIES.**—The leaves belonging to this group are the first ones produced by the propagating material after planting, as discussed in a preceding publication (6). At the time of harvest they were thoroughly mature and a few of them had become senile. Figure 6-A, containing the results of the chemical analyses of the different sections, shows that ammonium was present only in traces, while nitrate occurred in great amounts in the non-chlorophyllous and sub-chlorophyllous sections ( $C_1$  and  $C_2$ ). In the chlorophyllous sections the quantities of nitrate dropped very suddenly to low values. Soluble organic nitrogen values were intermediate and approximately of the same magnitude as those found in other studies (6). The fluctuations in the different nitrogenous fractions of the various sections cannot be satisfactorily explained in all cases. Protein values were comparatively high, indicating that conditions for its synthesis were favorable.

**NITRATE SERIES.**—Figure 6-B, illustrating the results of the chemical analyses of the different leaf sections, shows that the amounts of ammonium were very small, while those of nitrate were very great. Nitrate was restricted to the basal non-chlorophyllous sections, whereas ammonium, although occurring in very small amounts, was distributed in approximately uniform amounts in the different sections. The nitrate values for the non-chlorophyllous sections were considerably higher in this lot of plants than in the

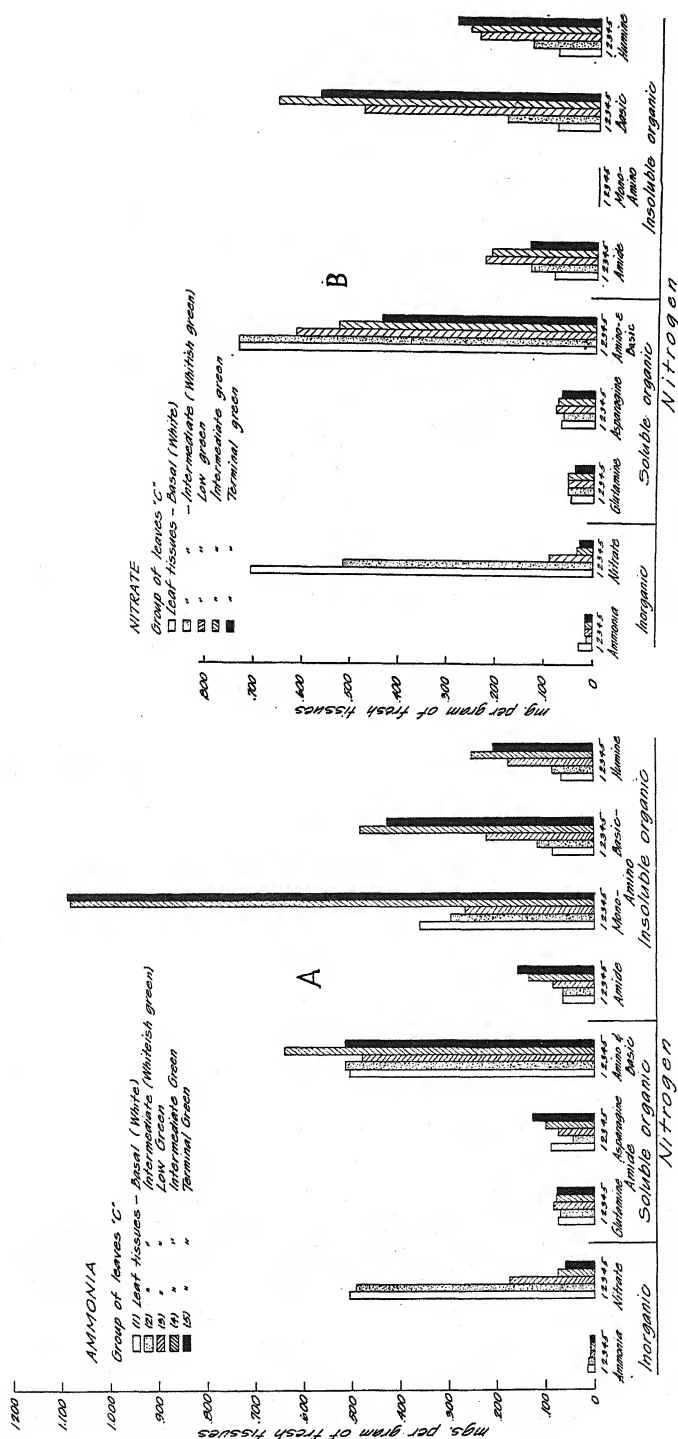


Fig. 6-A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

Fig. 6-B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (C) mature group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.



preceding one, indicating that applications of nitrate salts increased the nitrate content of plant tissues more rapidly than did the application of ammonium salts. Ammonium may be absorbed by the roots either as ammonium, or as nitrate, after oxidation to this latter form. When absorbed in the former state, only the values for the organic nitrogen fractions rise to high levels, while those for inorganic nitrogen, and especially for ammonium, remain very low. The amounts of the combined amino and basic fractions of soluble nitrogen were appreciably greater in the basal than in the intermediate and terminal sections of the leaves. The amounts of the two amide fractions (glutamine and asparagine) of soluble nitrogen fluctuated within a very narrow range in the different sections. The values for practically all the fractions of protein were low in the basal but high in the intermediate and terminal sections of the leaves. When the high figures for soluble organic nitrogen and low figures for protein in the basal sections are contrasted with the low content of soluble organic nitrogen and the high concentration of protein of the intermediate and terminal sections, they show that, as the values for soluble organic nitrogen decrease, those for protein increase. The low values for basic nitrogen in the  $C_s$  section (tip of old leaves) have possibly been obtained as a result of hydrolytic processes, which often develop in senile tissues.

#### ACTIVE LEAVES (D-GROUP)

**AMMONIUM SERIES.**—The D-group is composed of the longest, fully expanded and possibly most active leaves of the entire plant (6). The analytical data as presented in figure 7-A show that the amounts of ammonium were very small in all plant sections. The amounts of ammonium nitrogen which occur ordinarily in pineapple plant tissues are small regardless of the form of inorganic nitrogen applied to the plants. The ammonium found probably represents quantities derived from hydrolyzed native protein or from nitrate reduction. The amounts of nitrate were very high in the non-chlorophyllous basal sections but considerably smaller in the intermediate and terminal-chlorophyllous sections. This condition is characteristic of pineapple plants absorbing nitrate from a substratum.

Soluble organic nitrogen was found in greater quantities in the non-chlorophyllous basal than in the intermediate-chlorophyllous section of the leaves. The quantities in the mature terminal sections of the leaves rose to considerably greater values than in the intermediate ones, possibly either on account of retarded protein synthesis or of protein hydrolysis in the relatively senile tissues concerned.

With respect to the distribution of protein nitrogen, the amounts of different fractions of this form of nitrogen were low in the meristematic white section but high in the terminal-chlorophyllous sections. The values

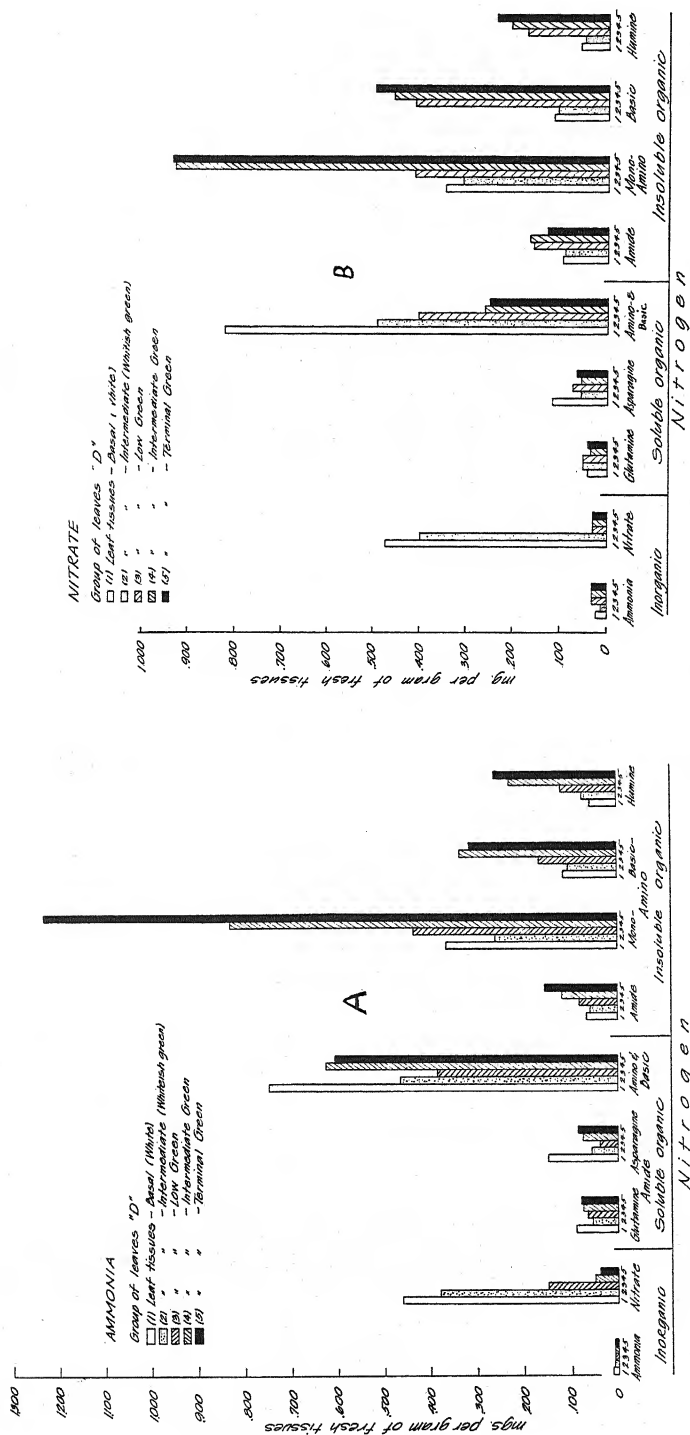


Fig. 7-A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

Fig. 7-B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

obtained harmonize well with those of the older leaf group C of the ammonium series.

**NITRATE SERIES.**—The amounts in grams of inorganic nitrogen as presented in figure 7-B show low values for ammonium and high values for nitrate. The values for ammonium were found to fluctuate in the different sections between 0.018 and 0.035 mg. while those for nitrate were between 0.032 and 0.474 mg. The high values for nitrate nitrogen were always associated with the non-chlorophyllous and the low with the chlorophyllous sections, as has been found in former cases. The combined fractions of amino and basic nitrogen were very high in the non-chlorophyllous basal sections and comparatively low in the chlorophyllous terminal ones. This condition did not develop in the same group of leaves in the ammonium series. In this series the values for amino and basic nitrogen decreased in the intermediate sections but increased in the terminal ones. Asparagine nitrogen was higher in the non-chlorophyllous than in the chlorophyllous sections, a condition which was also obtained in the corresponding group of leaves in the ammonium series.

The values for fractions of protein nitrogen were low in the non-chlorophyllous basal sections. In the intermediate- and terminal-chlorophyllous sections they rose gradually to high levels. The mono-amino fractions of protein nitrogen were found in nearly all cases to be lower in the plants supplied with nitrate than in plants supplied with ammonium salts, while the opposite was true with the basic fraction.

#### YOUNG LEAVES (E-GROUP)

**AMMONIUM SERIES.**—The E-group is composed of the youngest and most actively growing leaves of the plant (6). The analytical data presented in figure 8-A show that the quantities of ammonium were very low in all sections. Those of nitrate of the non-chlorophyllous sections, although high, are nevertheless considerably lower than in corresponding sections of the mature D and older C groups of leaves. This condition has been attributed (6) to an incomplete development of the fibrovascular bundles in the young apical section of the stem whereon the bases of these leaves are attached.

The amounts of the combined fractions of amino and basic soluble nitrogen were high in the non-chlorophyllous basal sections but low in the chlorophyllous terminal ones. The values for asparagine nitrogen are of the same order as those for the fraction of amino and basic nitrogen. The small amounts of the soluble organic nitrogen fractions in the chlorophyllous sections probably indicate a condition of rapid protein synthesis.

The amounts of the different fractions of protein nitrogen were slightly lower in the intermediate than in the basal and terminal sections. Humin nitrogen, regardless of leaf group or original source of nitrogen supply, was lowest in the basal and highest in the terminal sections of the leaves.

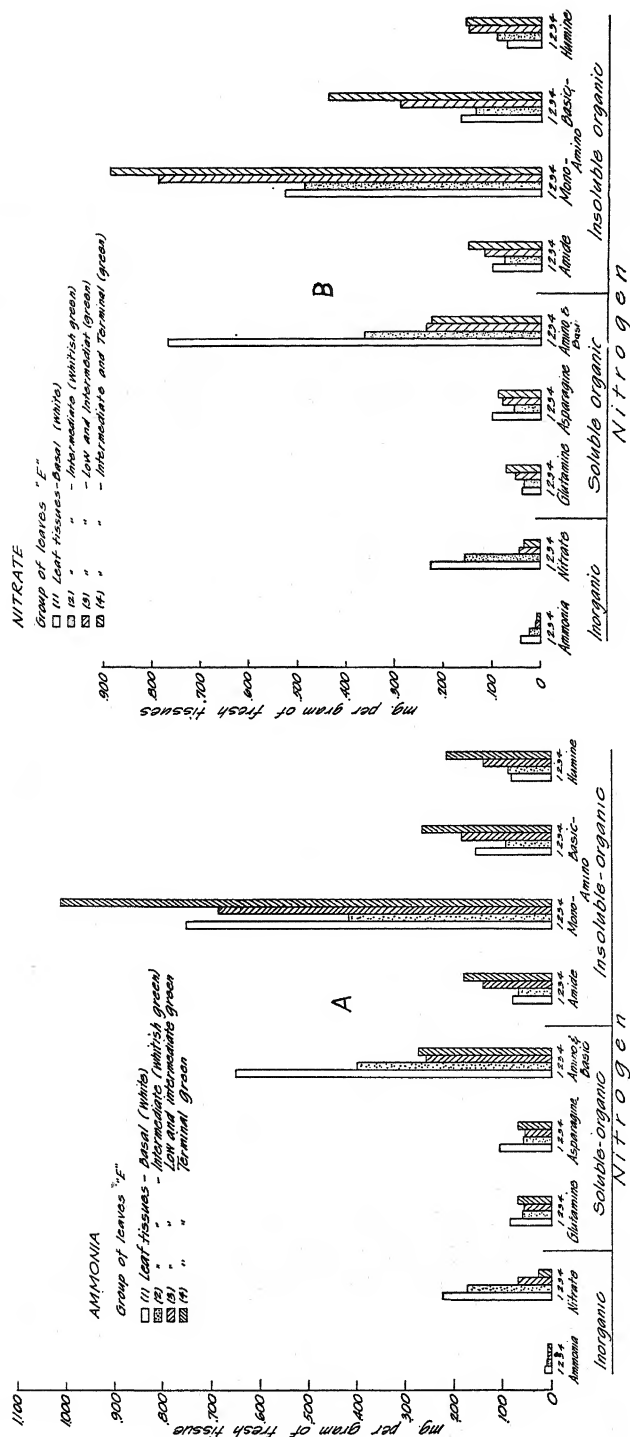


FIG. 8-A. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied with an ammonium salt as the source of nitrogen.

FIG. 8-B. Distribution of different fractions of inorganic, soluble organic and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

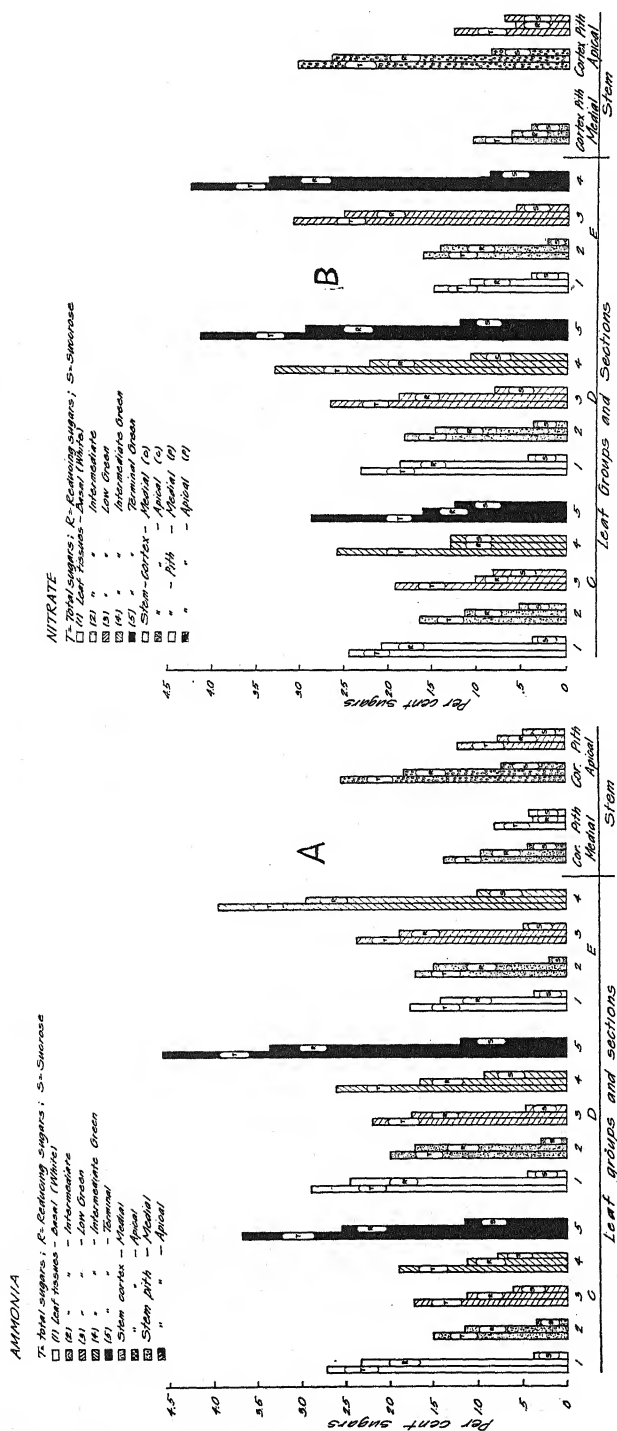
**NITRATE SERIES.**—Figure 8-B, presenting the analytical results for the various leaf sections of the nitrate series, shows that the amounts of ammonium nitrogen were low, while those of nitrate nitrogen were comparatively high. The quantities of nitrate in the basal sections of the leaves of this group were considerably less than in the same sections of the former two leaf groups. An explanation of this condition was offered above. The values are of the same magnitude as those for the corresponding leaf group of the ammonium series. The consistently higher values for ammonium in the various sections of the leaves from plants receiving nitrate as compared with those for sections of leaves from plants receiving ammonium nutrition cannot be explained other than on the assumption that they either represent reduced, but not assimilated nitrate, or hydrolyzed protein. The amounts of the combined fractions of amino and basic soluble nitrogen were higher in the non-chlorophyllous basal sections than in the chlorophyllous ones, a condition which is quite similar to the one observed in the plants receiving ammonium nutrition. The values of the two fractions of amide nitrogen (glutamine and asparagine) also had approximately the same order of distribution as they did in the other lot of plants.

The different fractions of protein were present in expected quantities, *i.e.*, their values were moderate in the basal, high in the terminal, and low in the intermediate sections. The humin nitrogen fraction was an exception and was present in quantities similar to those mentioned in the description of the preceding series. The basic nitrogen fraction of protein was higher in the nitrate than in the ammonium series of plants.

#### DISTRIBUTION OF SUGARS

The analytical data presented in table VI and figures 9-A and 9-B show the distribution of total, reducing sugars, and sucrose, the latter representing the difference between the two former values. No other kinds of carbohydrates were determined on account of accidental spoilage of the dried tissues.

**AMMONIUM SERIES.**—Figure 9-A, representing the amounts of the above mentioned sugars found in the different sections of the ammonium lot of plants, shows that the terminal sections contained, in general, more sugars than the intermediate ones. The basal sections in well developed and mature leaves contained greater amounts of sugars than some of the intermediate sections in the same group. In young leaves, however, the amounts of sugars in the basal sections were relatively low, presumably because they were used in tissue building. The accumulation of sugars in the basal sections of well grown and mature leaves is the result of either a decreased rate in the processes of building new tissue or a result of impeded translocation; or, a result of both of these acting simultaneously. The amounts of sugars



**Fig. 9-B.** Distribution of reducing and total sugars and sucrose in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (O) mature, (D) active, and (E) young leaves, and in (C) the cortex and (P) pith of the medial and apical sections of the stem of plants grown in soil and supplied with a nitrate salt as the source of nitrogen.

TABLE VI

DISTRIBUTION OF TOTAL SUGARS, REDUCING SUGARS, AND SUCROSE IN DIFFERENT SECTIONS OF THE LEAVES AND STEM OF PINEAPPLE PLANTS GROWN IN THE SOIL AND RECEIVING EITHER AMMONIUM OR NITRATE NITROGEN

PLANT SECTIONS	NUTRITION.. SERIES					
	AMMONIA			NITRATE		
	SUGARS			SUGARS		
	TOTAL	REDUCING SUGARS	SUCROSE	TOTAL	REDUCING SUGARS	SUCROSE
Leaves	%	%	%	%	%	%
Mature						
C1 (Base)	2.73	2.34	0.39	2.45	2.08	0.37
C2 .....	1.49	1.15	0.34	1.64	1.13	0.51
C3 .....	1.72	1.12	0.60	1.92	1.01	0.81
C4 .....	1.91	1.14	0.77	2.58	1.29	1.29
C5 (Tip)...	3.67	2.54	1.13	2.86	1.61	1.25
Active						
D1 (Base)	2.91	2.47	0.44	2.31	1.88	0.43
D2 .....	2.00	1.71	0.29	1.83	1.47	0.36
D3 .....	2.21	1.75	0.46	2.66	1.87	0.79
D4 .....	2.61	1.67	0.94	3.30	2.22	1.08
D5 (Tip)...	4.56	3.36	1.20	4.14	2.94	1.20
Young						
E1 (Base)	1.78	1.42	0.36	1.49	1.09	0.40
E2 .....	1.70	1.50	0.20	1.63	1.41	0.22
E3 .....	2.36	1.88	0.48	3.10	2.52	0.58
E4 (Tip)...	3.93	2.94	0.99	4.25	3.37	0.88
Stem						
Medial						
Cortex .....	1.37	0.94	0.43	1.06	0.64	0.42
Pith .....	0.80	0.37	0.43	.....	.....	.....
Apical						
Cortex .....	2.52	1.80	0.72	3.05	2.17	0.88
Pith .....	1.22	0.75	0.47	1.31	0.59	0.72

were greater in the sections of the D-group of leaves than in those in either the C- or E-groups, indicating that protoplasmic vigor and a retarded rate of the processes of building new tissue were responsible for the greater amounts of sugars. Although protoplasmic vigor was as great and possibly greater in the cells of the young E-group of leaves, the smaller amounts of sugars present were due to a very great rate of synthesis of new tissues. Partial lack of protoplasmic vigor may be the cause of the relatively smaller amounts of sugars found in the sections of the oldest, or C-group, as compared to those found in the fully expanded but very active D-group of leaves. The order of distribution of sucrose was about the same in all three groups of leaves. The amounts in the terminal sections were from 2 to 3 times greater than in the basal ones, section 2 having the lowest value in all leaf groups.

The amounts of sugars in the cortex and pith of the stem were very

unequally distributed. The cortex contained two and one-half times more reducing sugars than the pith. The greater amounts of sugars in the cortex were expected because of the direct connection of this tissue with the leaf bases. The smaller amounts of sugars in the pith were possibly attributable either to a rapid conversion of substances to starch, or to a decreased rate of translocation. The young apical section of the stem contained greater amounts of sugars than the medial section, possibly because of the attachment to it of leaf groups D and E, which are more efficient sugar producers as a result of their greater protoplasmic vigor, than leaves of group C which are relatively senescent.

NITRATE SERIES.—The order of distribution of sugars in the different sections of the plants of the nitrate series was about the same as in the ammonium group. There were, in some cases, slight differences but none were sufficiently great to be of significance. The comments presented in the preceding case apply equally well to this one.

### Discussion

The analytical data presented in the foregoing studies have indicated that the chemical composition of plants grown in non-sterilized soil cultures and supplied with either ammonium or nitrate salts were, with a few exceptions, about the same. The exceptions were in the values for the water content of the tissues which were slightly greater for the ammonium than for the nitrate lot of plants. One may state also, by analogy, that the amounts of organic matter per gram of fresh weight were smaller in the ammonium than in the nitrate lot of plants. Regardless of how this condition is stated, the facts are that, with ammonium nutrition, the percentage of organic matter produced per unit of fresh weight is considerably smaller than with nitrate nutrition. Some differences found in the electrical resistance of the extracted sap of corresponding leaf and stem sections of the two lots of plants were slightly greater in the nitrate than in the ammonium series. The titratable acidity of the expressed sap was considerably greater in the plants of the nitrate than in those of the ammonium series. The greater moisture content of the plants of the ammonium series has been discussed in certain other studies (6). With respect to the greater values of electrical resistance, no satisfactory explanation can be offered until complete data are obtained on the chemical composition of the sap. The greater acidity values in the plants of the nitrate series cannot be explained without additional experimental data.

The distribution of the different nitrogen fractions in the various sections of the leaves and stem of the two lots of plants was, with minor exceptions, about the same. In our former studies (6), where plants were grown in solution cultures instead of soil, the chemical composition of the plants



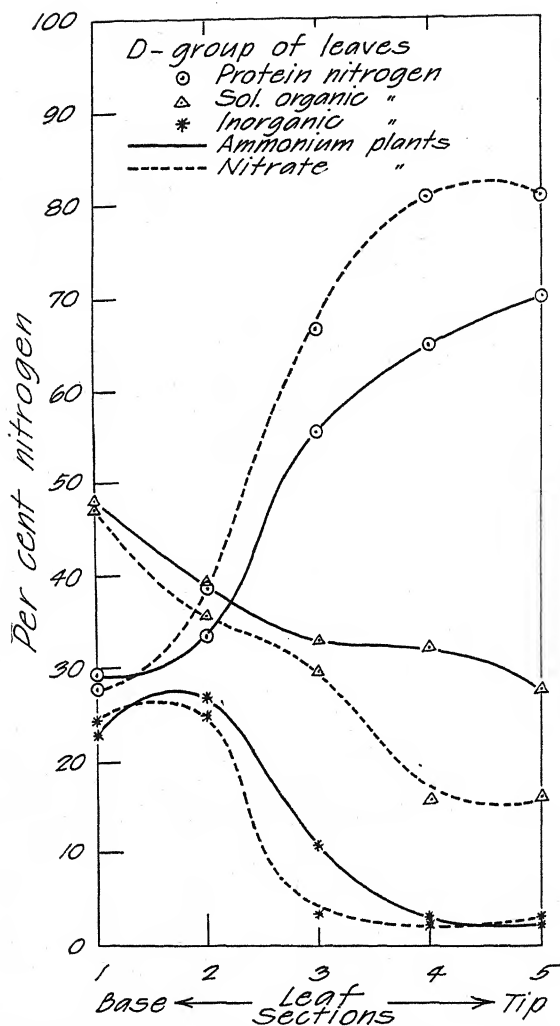


FIG. 10. Percentage of total inorganic, soluble organic, and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (D) the active group of leaves of plants grown in soil and supplied either with an ammonium or with a nitrate salt as the source of nitrogen.

receiving ammonium nitrogen differed considerably from that of the plants supplied with nitrate. Such differences have been slight in the two lots of plants grown in soil because of the rapid oxidation in the soil of ammonium to nitrate and the subsequent simultaneous absorption of both ions by the plants. The presence of nitrate in the non-chlorophyllous basal sections of the leaves and in the stem of the plants receiving ammonium salts is ample

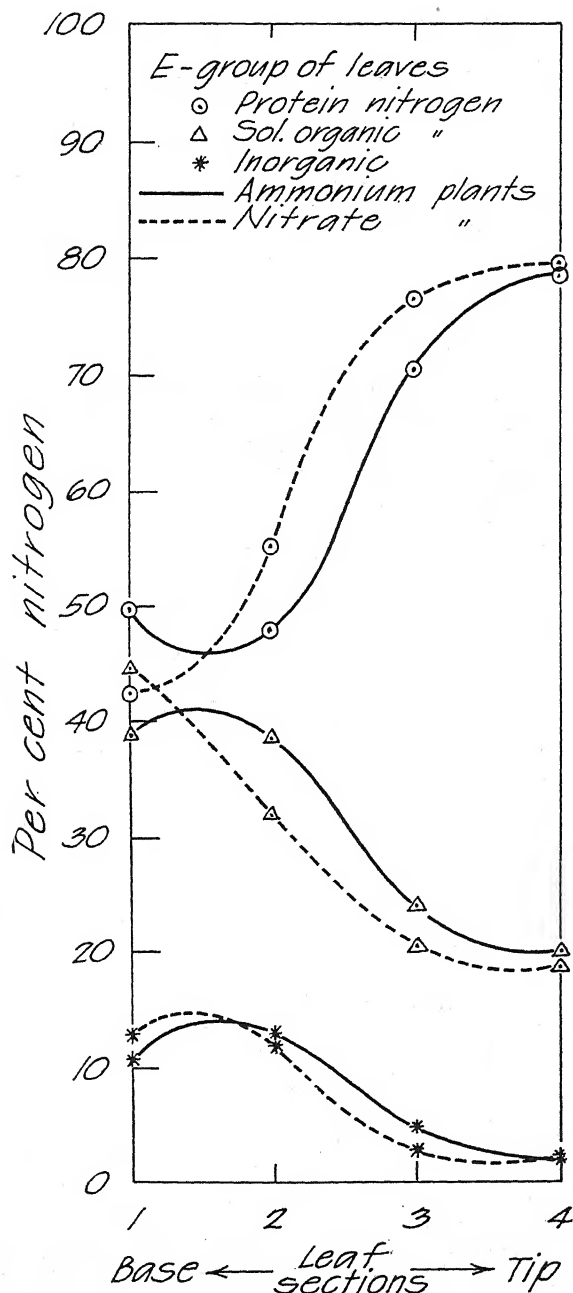


FIG. 11. Percentage of total inorganic, soluble organic, and insoluble organic nitrogen in (1) the basal, non-chlorophyllous; (2) transitional, sub-chlorophyllous; (3) lower; (4) intermediate; and (5) terminal-chlorophyllous sections of (E) the young group of leaves of plants grown in soil and supplied either with an ammonium or with a nitrate salt as the source of nitrogen.

TABLE VII

ILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT SECTIONS TOGETHER WITH PERCENTAGE VALUES OF THE SAME AND OF THEIR FRACTIONS AS FOUND IN PINEAPPLE PLANTS GROWN IN SOIL CULTURES RECEIVING AMMONIUM NITROGEN

PLANT SECTIONS	INORGANIC				SOLUBLE ORGANIC				INSOLUBLE ORGANIC							TOTAL OR- GANIC N	TOTAL N
	PERCENTAGE OF INORGANIC		TOTAL	PER- CENT- AGE OF TOTAL	PERCENTAGE OF SOLUBLE ORGANIC			TOTAL	PER- CENT- AGE OF TOTAL	PERCENTAGE OF INSOLUBLE ORGANIC				TOTAL	PER- CENT- AGE OF TOTAL		
	NH <sub>4</sub>	NO <sub>3</sub>			GLUTA- MINE	AS- PARA- GINE	REST N			AMIDE N	MONO- AMINO N	BASIO N	HU- MIN N				
Leaves	%	%	mg.	%	%	%	%	mg.	%	%	%	%	%	mg.	%	mg.	
Mature																	
C1 (Base)	2.8	97.2	0.502	28.9	11.2	13.3	75.5	0.668	38.3	10.9	62.7	14.7	11.7	0.571	32.8	1.239	1.741
C2	1.9	98.1	0.516	30.4	11.1	7.3	81.6	0.630	37.0	11.2	53.5	20.2	15.1	0.555	32.6	1.185	1.701
C3	4.8	95.2	0.187	11.9	13.7	11.2	75.1	0.640	40.7	10.9	35.5	29.6	24.0	0.743	47.4	1.383	1.570
C4	11.6	88.4	0.086	3.0	10.0	12.2	77.8	0.825	28.9	7.0	55.5	24.7	12.8	1.946	68.1	2.771	2.857
C5 (Tip)	11.7	88.3	0.077	2.9	11.2	17.7	71.1	0.723	27.1	8.3	58.0	22.8	10.9	1.860	70.0	2.583	2.660
Active																	
D1 (Base)	2.8	97.2	0.473	23.0	9.1	15.2	75.7	0.990	48.0	11.2	60.8	18.9	9.1	0.599	29.0	1.589	2.062
D2	2.0	98.0	0.391	26.7	9.3	10.2	81.5	0.581	39.7	12.0	52.4	20.9	14.7	0.493	33.6	1.074	1.465
D3	6.7	93.3	0.164	11.3	13.2	8.5	78.3	0.485	33.3	10.7	53.6	20.9	14.8	0.806	55.4	1.291	1.455
D4	17.2	82.8	0.070	2.9	9.2	9.7	81.1	0.774	32.8	7.9	54.7	25.0	12.4	1.514	64.3	2.288	2.358
D5 (Tip)	14.7	85.3	0.068	2.5	9.9	11.2	78.9	0.767	27.5	7.9	62.8	16.1	13.2	1.960	70.0	2.727	2.795
Young																	
E1 (Base)	5.1	94.9	0.236	11.0	10.0	12.4	77.6	0.838	39.2	7.5	70.7	14.5	7.3	1.064	49.8	1.902	2.138
E2	5.0	95.0	0.182	13.2	11.5	11.5	77.0	0.537	38.8	10.5	62.4	14.4	12.7	0.665	48.0	1.202	1.384
E3	11.4	88.6	0.079	5.0	16.2	15.4	68.4	0.377	24.2	8.4	62.1	16.8	12.7	1.102	70.8	1.479	1.558
E4 (Tip)	25.5	74.5	0.039	1.8	17.1	16.6	66.3	0.415	20.0	8.2	62.4	16.3	13.1	1.621	78.2	2.038	2.075
stem																	
Medial																	
Cortex C	10.1	89.9	0.426	15.1	6.7	10.7	82.6	1.488	52.7	14.2	49.3	19.3	17.2	0.910	32.2	2.398	2.824
Pith C	10.5	89.5	0.492	15.8	5.0	10.3	84.7	1.841	59.0	12.1	52.8	21.6	13.5	0.786	25.2	2.627	3.119
Apical																	
Cortex D	11.9	88.1	0.302	11.0	8.6	13.3	78.1	1.498	54.4	11.0	65.4	13.2	10.4	0.954	34.6	2.452	2.754
Pith D	11.2	88.8	0.400	11.9	8.4	16.4	75.2	1.996	59.4	12.0	60.5	17.4	11.1	0.966	28.7	2.962	3.362

PLANT SECTIONS	INORGANIC			SOLUBLE ORGANIC					INSOLUBLE ORGANIC					TOTAL OR-GANIC N	TOTAL N
	PERCENTAGE OF INORGANIC		PER-CENT-AGE OF TOTAL	PERCENTAGE OF SOLUBLE ORGANIC			PER-CENT-AGE OF TOTAL	PERCENTAGE OF INSOLUBLE ORGANIC				TOTAL	PER-CENT-AGE OF TOTAL		
	NH <sub>4</sub>	NO <sub>3</sub>		GLUTA-MINE	AS-PARA-GINE	REST N		AMIDE N	MONO-AMINO N	BASIC N	HU-MIN N				
Leaves	%	%	mg.	%	%	%	%	mg.	%	%	%	mg.	%	mg.	
Mature															
C1 (Base)	4.0	96.0	0.731	3.1	8.3	86.6	0.847	47.8	15.6	56.0	19.0	0.626	27.7	1.544	
C2	1.9	98.1	0.524	5.5	8.1	86.4	0.850	36.0	13.8	47.5	30.6	0.653	39.0	1.254	
C3	9.8	90.2	0.102	6.5	11.0	82.5	0.742	29.9	13.6	35.6	35.8	1.162	66.5	1.748	
C4	22.8	77.2	0.044	7.4	11.6	81.0	0.645	15.8	9.1	53.0	26.1	1.759	81.2	2.103	
C5 (Tip)	32.0	68.0	0.050	7.3	14.5	78.2	0.565	16.2	7.2	51.8	27.8	1.798	80.5	2.234	
Active															
D1 (Base)	5.4	94.6	0.501	3.9	11.8	84.3	0.977	47.8	15.6	56.0	19.0	0.626	27.7	1.544	
D2	4.3	95.7	0.418	8.3	9.7	82.0	0.601	36.0	13.8	47.5	30.6	0.653	39.0	1.254	
D3	49.0	51.0	0.063	9.6	13.4	77.0	0.523	29.9	13.6	35.6	35.8	1.162	66.5	1.685	
D4	45.5	54.5	0.066	9.3	16.0	74.7	0.344	15.8	9.1	53.0	26.1	1.759	81.2	2.103	
D5 (Tip)	47.5	52.5	0.074	10.5	18.0	71.5	0.362	16.2	7.2	51.8	27.8	1.798	80.5	2.234	
Young															
E1 (Base)	17.9	82.1	0.264	4.4	11.2	84.4	0.910	44.6	11.6	61.4	19.2	0.863	42.4	1.773	
E2	13.0	87.0	0.177	7.1	12.0	80.9	0.451	31.9	9.3	62.4	16.9	0.785	55.5	1.236	
E3	18.9	81.1	0.053	13.7	22.1	64.2	0.366	20.6	8.5	57.8	21.6	1.348	76.4	1.714	
E4 (Tip)	34.7	65.3	0.049	18.1	21.9	60.0	0.392	19.0	9.1	54.6	27.0	1.629	78.7	2.070	
Stem															
Medial															
Cortex C	3.5	96.5	1.014	3.3	11.1	85.6	1.670	44.8	9.7	57.0	18.9	1.039	27.9	2.709	
Pith C	3.2	96.8	1.099	2.7	11.2	86.1	2.001	49.2	9.9	58.3	20.6	0.960	23.7	2.961	
Apical															
Cortex D	9.7	90.3	0.718	6.1	20.0	73.9	1.454	44.1	10.2	59.0	21.8	1.131	34.2	2.585	
Pith D	3.2	96.8	0.986	5.0	21.4	73.6	1.753	46.4	11.9	61.2	17.2	1.046	27.6	2.799	

evidence of the oxidation of ammonium to nitrate. Both lots of plants contained great amounts of nitrate and soluble amino and basic nitrogen which, on the basis of our previous studies (6), indicate that both ammonium and nitrate ions were possibly absorbed simultaneously from the soil. In the absence of analytical data on plants receiving both ammonium and nitrate ions in solution cultures, we cannot explain other differences between the two lots of plants. Tables VII and VIII, presenting a more comprehensive analysis of the data in table V, show the following conditions:

1. That the percentage of total nitrogen of the stem was considerably greater in the nitrate than in the ammonium series. The percentage of nitrogen of the leaves was either of the same magnitude or slightly greater in the ammonium than in the nitrate series. The percentage of total nitrogen of the plants in the ammonium series was less when grown in soil than in solution cultures (6). It is possible that this condition might have been the result either of the oxidation of ammonium to nitrate which decreased the rate of absorption, on the basis of previous data (6), or possibly of the greater weight of the ammonium lot of plants, as compared with that of the nitrate lot, attained in soil cultures.

2. The percentage values of protein nitrogen as shown in figures 10 and 11 are higher for the plants receiving nitrate than for the plants receiving ammonium nitrogen. For the soluble organic nitrogen content, however, the values are reversed, except in the non-chlorophyllous basal sections. Inorganic nitrogen percentage values are slightly greater in the ammonium than in the nitrate series with the exception of the non-chlorophyllous basal section.

3. The sugar values in the different sections of the two lots of plants present no outstanding differences but the relatively high percentage of dry matter in the plants supplied with nitrate necessarily means greater carbohydrate synthesis although, excepting sugars, no other carbohydrates were determined.

In view of the above consideration of the experimental data, no outstanding differences can be assigned to the chemical composition of the tissues of plants grown in non-sterilized soil cultures and receiving either ammonium or nitrate salts as sources of nitrogen. The lack of such differences was possibly due to the oxidation in the soil of ammonium to nitrate.

Comparing the results of these studies with those obtained previously, one may safely state that a very great portion of the ammonium of salts which had been added to the soils, was rapidly converted to nitrate and then was absorbed as such by the plants. That some ammonium was apparently absorbed in its native state is suggested by the greater percentage values for the combined fractions of amino and basic nitrogen in the chlorophyllous sections of leaves of the groups C and D of the ammonium series than for

the nitrate series of plants. This could well be the result of ammonium absorption (6).

### Summary

Pineapple plants grown in non-sterilized soil cultures and receiving either ammonium or nitrate salts as sources of nitrogen weighed more when grown in the cultures receiving ammonium than in those receiving nitrate.

The results of the analyses of leaf and stem sections indicated that the chemical composition of the different sections of the leaves and stem of the plants of both series, with minor exceptions, was about the same.

The most outstanding feature of these studies was the abundance of nitrate in the stem and in the non-chlorophyllous basal leaf tissues of the leaves of plants supplied with ammonium. Similar plants grown previously in nitrate-free solution cultures with ammonium salts contained no nitrate in their tissues but great amounts of amino nitrogen. The nitrate found in the tissues of the plants supplied with ammonium in the present studies was derived from ammonium which had been oxidized in the soil by microorganisms. When nitrate is absorbed by the roots from solution cultures it is transported in its native state through the tissues of the roots and stem to the chlorophyllous parts of the leaves wherein it is readily assimilated. The oxidation of ammonium salts added to soils is indicated by the presence of abundant nitrate in the non-chlorophyllous tissues of the plants.

The protein content of the leaves of plants of the nitrate series was slightly greater than that of those of the ammonium group. The soluble organic nitrogen was found to be higher, however, in the plants of the ammonium than in those of the nitrate series.

As ammonium is assimilated very quickly, inorganic nitrogen consisted mostly of nitrate; it was high in the stem and in the non-chlorophyllous sections of the leaves of the plants of both series of soil cultures.

Contrasting these findings with those of preceding studies it is safe to state that the chemical composition of plants grown in non-sterilized soil and receiving either ammonium or nitrate salts as sources of nitrogen varies very little, because of the conversion of ammonium to nitrate by the nitrifying bacteria of the soil.

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# EFFECT OF TEMPERATURE UPON THE RATE OF ELONGATION OF THE STEMS OF ASPARAGUS GROWN UNDER FIELD CONDITIONS

C. W. CULPEPPER AND H. H. MOON

(WITH FIVE FIGURES)

## Introduction

In connection with investigations on the use of asparagus (*Asparagus officinalis* Linn.) as a food product (2), an opportunity was afforded to study some of the factors that influence or control its growth. All tests were confined to field-grown material. Since the effect of temperature upon the rate of growth of asparagus in the field is of considerable practical as well as physiological interest, it was decided to make a sufficient number of measurements to permit accurate determination of the relationship of the rate of elongation of the stem or stalk to the prevailing temperatures. In general, the method has been to study the behavior of a large number rather than that of individual plants. The procedure employed was to record the climatic variations that occurred and the behavior of the plants under these conditions, and then to correlate the values obtained in the two sets of records, a method frequently employed by investigators.

Studies have been made upon the rate of growth of the stem of asparagus by BÜCHNER (1), TIEDJENS (9), WICHERS and TOLLENS (11), and WORKING (12) but no very thorough study has been made of the effect of temperature upon the behavior of field material in mass.

## Materials and methods

The material employed in these studies was grown at the Arlington Experiment Farm, Arlington, Virginia, during the seasons 1928, 1932, and 1933. The plot, approximately  $\frac{1}{16}$  of an acre, had been planted in 1919, so that when the first tests were made, the planting was nine years old. The soil was a deep sandy loam of good fertility formed by dredging operations along the Potomac River. The plants were vigorous and healthy, and generally continued growth until they were 230 to 250 cm. in height. The variety was a strain of Martha Washington used in some of the breeding and selection work conducted in the U. S. Department of Agriculture.

The air temperature of the field was recorded in °F. by means of a carefully calibrated thermograph placed in a Weather Bureau instrument shelter in the center of the plot. Records were kept for the entire cutting season from about April 1 to June 15, and as usual, fluctuations in the temperature were rather wide and irregular. The lowest temperature recorded was approximately 45° F. and the highest 94° F. The temperatures given in the



tables and charts for any growth period are the hourly means for the respective periods.

#### METHODS OF MEASURING GROWTH

The rate of growth was determined by direct measurement of stalk height, using a rule graduated in mm. A small stake was driven into the soil beside the stalk so that its upper end was about 2.5 cm. below the surface of the soil, and all measurements of growth of the stalk were made from the top of this stake as a base. The measurements were begun at the time, or very soon after, the stalks appeared above ground and in most cases were continued until growth had practically ceased. The measurements were usually made once each day at approximately 9:30 A.M. In a number of cases an additional reading was made at about 4:00 P.M. All stalks of the plot not being measured were cut at regular intervals as in commercial practice. Cutting was discontinued May 17, while in commercial practice it is usually continued until about June 15.

The measurements were begun on April 20, or shortly after the first stalks began to appear above the ground, and continued until the latter part of June.

The total elongation of the stalks for any period was determined by taking the difference in height at the beginning and at the end of the time interval. The height for the growth interval was calculated as the average of the height at the beginning and at the end of the interval.

To determine the rate of elongation in different regions, marks were made with a small brush and waterproof ink at suitable intervals on the bases of the leaf scales, generally from 0.5 to 1 cm. apart, in the rapidly growing part of the stalk. The increase in the length of the segments thus marked was determined for successive time intervals. The elongating part of the stalk was divided into 5 to 15 segments, the number in each case depending upon the length of the growing region. As the stalks increased in height new marks were made from time to time near the tip to keep the intervals of measurement appropriately located along the growing zone. The method was essentially that originally employed by SACHS (8) in his studies of the grand period of growth of plants.

At the beginning of the season measurements were started on about a dozen stalks which were identified by means of numbered tags. Every second or third day thereafter 2 to 5 new stalks just showing above the ground were tagged and measurements begun. Soon stalks of all heights were being measured at every period. In all, several thousand measurements were made upon a total of 130 stalks.

#### METHODS OF ANALYZING AND PLOTTING DATA ON GROWTH RATES

**TOTAL ELONGATION OF THE STALK.**—It was obvious from inspection of the data that individual stalks varied considerably in the ultimate height reached

and in their rate of elongation. In order to determine the general behavior and to get the mean values, it appeared to be advantageous to employ a system of cross classification. The data were first classified according to the prevailing temperatures of the growth periods. Five-degree class intervals were established, extending over the range from 50° to 90° F. This gave eight temperature groups.

Inspection showed that each group included data for stalks of all heights and that the rate of elongation at a given temperature was different in stalks of different heights. The measurements in each temperature group were then classified according to the height of the stalk, as follows: All values for the growth of stalks below 10 cm. in height were put into one class, those between 10 and 20 cm. were put into another class, and so on at 10 cm. intervals until stalks 80 cm. in height were reached; then, 20 cm. classes were used up to 200 cm. in height. The values in each class were then averaged and tabulated in a manner similar to that of table I. There were 14 classes for each of the eight temperature groups, making 112 classes in all. The number of measurements in each of the classes ranged from 10 to 64. The smallest number of values in any class was for the taller stalks at the lower temperatures.

These values were plotted in two ways: the rate of growth against the tem-

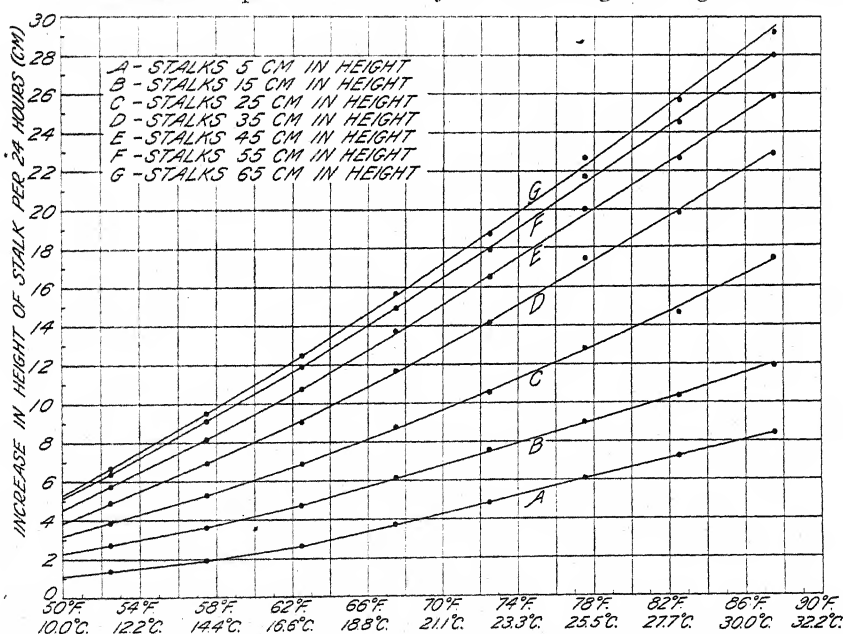


FIG. 1. Effect of temperature on the total elongation of asparagus stalks of various heights between 5 and 70 cm. The plants were grown under field conditions and the results are expressed as cm. growth per 24 hours. Average height of stalks for the 24-hour period.

TABLE I  
RATE OF GROWTH OF ASPARAGUS STALKS AT DIFFERENT HEIGHTS AND TEMPERATURES, EXPRESSED IN CM. OF ELONGATION PER 24 HOURS

AVERAGE TEMPERATURE	AVERAGE HEIGHT OF STALK (CM.)													
	5	15	25	35	45	55	65	75	90	110	130	150	170	190
	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
°F.	RATE OF GROWTH													
	1.35	2.6	3.9	4.9	5.8	6.4	6.6	6.3	5.5	4.5	3.5	2.65	2.1	1.55
	1.90	3.6	5.3	6.9	8.2	9.1	9.5	9.0	7.85	6.45	5.05	3.85	3.0	2.25
	2.60	4.7	6.9	9.1	10.75	11.9	12.4	12.5	10.75	8.8	6.95	5.25	4.0	2.95
	3.7	6.1	8.7	11.65	13.6	14.9	15.6	15.25	13.65	11.35	8.9	6.7	5.2	3.80
	4.8	7.4	10.6	14.2	16.5	18.0	18.8	18.6	16.85	13.9	10.85	8.15	6.3	4.7
	7.2	10.4	14.9	20.05	22.8	24.65	25.8	22.15	20.3	16.8	13.0	9.7	7.55	5.65
	8.4	12.0	17.45	22.95	25.95	28.0	29.35	29.0	26.9	22.45	17.4	13.1	10.4	7.85

perature, giving a series of 14 curves; and against the height of the stalk, giving a series of eight curves. The first method of plotting is shown in figures 1 and 2, and the second in figure 3.

When the growth rates were plotted against either temperature or height, the points did not fall exactly upon a smooth curve. By means of a spline, or curve rule, a smooth curve was drawn through the points in such a manner that the sum of the squares of the deviations was as nearly a minimum as could be estimated by simple arithmetical calculations.

The values were plotted upon coordinate paper upon a large scale. The new average or adjusted values of the rates of growth for the midpoints of each of these 112 classes were read from the chart for both systems of curves. Obviously, these values should be the same in both systems of curves but they were not identical in many cases, some being higher and some lower in one system than in the other. The two values for each of the corresponding points were averaged and the new values tabulated in the same way as was done at first. These new values were again plotted as had been done in the first place and new curves drawn in the same way. When the values were read from the new curves they agreed much more closely than at first. After plotting and averaging in this way three times, the values (table I) finally obtained, when plotted, form loci of points that lie almost exactly upon smooth curves. They represent estimated mean values of the growth rates of the stalks of asparagus in this plot as a whole.

**DIFFERENT REGIONS ALONG THE STALK.**—In order to avoid the presentation of an excessive amount of tabular matter, the data for rate of growth in different regions of the stalk were classified in the same manner as the data for the total elongation of the stalk. A particular stalk in each of these classes had, instead of one reading as in the values for the total elongation, a series of readings made at various points from base to tip. The values for the growth rates at corresponding points along the stalks of all the individuals in the class were averaged. This was done at a sufficient number of points (7 to 12) to permit plotting of the results. When these values were plotted, the line represented the rate of growth along the entire growing region for the stalks of a particular height at a particular temperature. Obviously, to plot the rates of growth for 112 classes would require a great amount of space. Consequently, the data for the growth rates of stalks of only two heights, 15 and 75 cm., have been calculated for the temperatures between 52.5° and 77.5° F. This is believed to be sufficient to illustrate the general character of the results.

### Observations

#### EFFECT OF TEMPERATURE UPON TOTAL ELONGATION

After making a few measurements it was obvious that there was a very definite positive correlation between the growth of asparagus and the prevail-

ing temperatures as shown in table I. The results for all plants 70 cm. or less in height are illustrated in figure 1, while those for plants more than 70 cm. in height are shown in figure 2. It is evident that the relationship between temperature and total elongation per 24 hours is in all cases represented by nearly straight lines. They are slight curves which slope upward a little more sharply in the portion of the curves representing the higher temperatures. This is true for stalks of all heights. The form of the curve

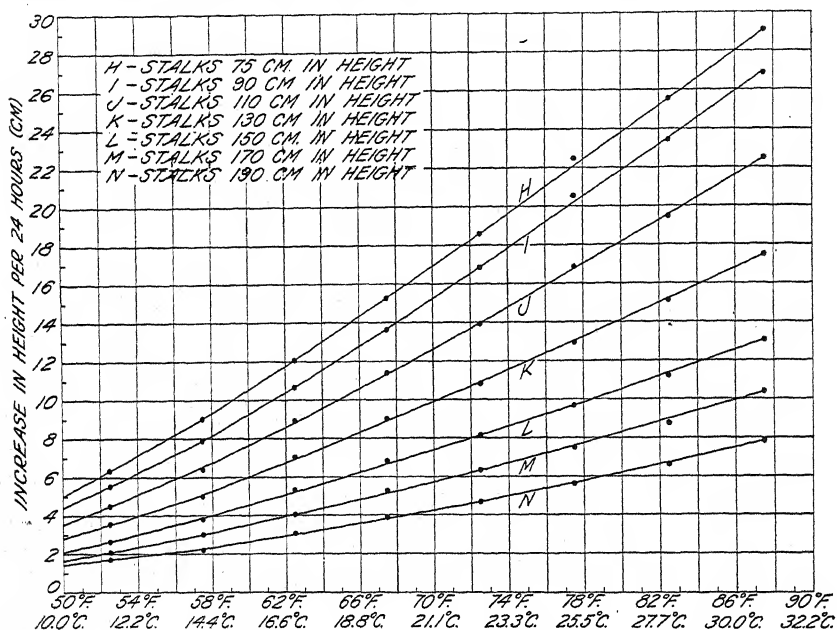


FIG. 2. Effect of temperature on the total elongation of asparagus stalks of various heights between 70 and 200 cm. The plants were grown under field conditions and the results are expressed as cm. growth per 24 hours. Average height of stalks for the 24-hour period.

is nearly the same whatever the height of the stalk may have been at the time of measurements. The difference in slope of the lines indicates that stalks 60 to 70 cm. high are a little more sensitive to changes in temperature than those that are taller or shorter. The reason for this is not clear but it seems to be associated with the length of the growing zone. Also the very old stalks are not quite so sensitive to increases in temperature as are the very young stalks. This may be due in part to failure to transport water and food materials to the growing region of the tall stalks at the higher temperatures as rapidly as the materials are required for the formation of new tissues. It would be of interest to have comparable data on material with the prevailing temperatures both higher and lower than those which prevailed under these

TABLE II

RATE OF GROWTH AT VARIOUS 10° C. INTERVALS AND THE RATIO OF THE RATE OF GROWTH AT THE END TO THAT AT THE BEGINNING OF THE INTERVAL

HEIGHT OF STALKS IN CM.

TEMPERATURE	5			25			65			130			190		
	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a	GROWTH RATE		RATIO b/a
	LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b		LOWER TEMP. a	UPPER TEMP. b	
°C.	cm.	cm.		cm.	cm.		cm.	cm.		cm.	cm.		cm.	cm.	
0 and 20	1.08	3.77	3.49	3.18	8.81	2.77	5.28	15.90	3.01	2.80	9.03	3.22	1.43	3.90	2.72
1 and 21	1.30	4.18	3.21	3.65	9.50	2.60	6.27	17.04	2.88	3.30	9.80	2.97	1.60	4.20	2.62
2 and 22	1.50	4.60	3.06	4.20	10.20	2.43	7.29	18.24	2.50	3.81	10.52	2.76	1.80	4.50	2.50
3 and 23	1.68	5.02	2.98	4.70	10.95	2.33	8.30	19.43	2.34	4.40	11.28	2.56	1.96	4.86	2.47
4 and 24	1.87	5.48	2.92	5.20	11.70	2.25	9.33	20.63	2.21	4.98	12.02	2.41	2.20	5.18	2.35
5 and 25	2.10	5.90	2.81	5.72	12.43	2.17	10.38	21.88	2.10	5.60	12.80	2.28	2.47	5.54	2.24
6 and 26	2.36	6.33	2.68	6.30	13.22	2.10	11.41	23.17	2.03	6.27	13.59	2.16	2.72	5.90	2.20
7 and 27	2.64	6.75	2.56	6.91	14.03	2.03	12.52	24.43	1.95	6.94	14.38	2.07	3.00	6.30	2.10
8 and 28	2.97	7.20	2.42	7.43	14.92	2.01	13.50	25.80	1.91	7.54	15.22	2.01	3.26	6.7	2.07
9 and 29	3.38	7.60	2.26	8.15	15.75	1.93	14.72	27.06	1.84	8.32	15.96	1.92	3.58	7.08	1.98
10 and 30	3.77	8.02	2.12	8.80	16.63	1.89	15.88	28.37	1.79	9.04	16.74	1.84	3.90	7.48	1.92
11 and 31	4.18	8.50	2.03	9.51	17.6	1.84	17.04	29.60	1.73	9.80	17.55	1.79	4.20	7.90	1.88

conditions. By extrapolation of the curves it may be inferred that below 40° F. very little or no growth will occur. It is also evident that at 87.5° F. (30.8° C.), the highest average temperature recorded, the rate of elongation had not reached its maximum. From the slope of the curves it appears unlikely that under field conditions, temperatures high enough for maximum rate of elongation will often prevail in this latitude.

In order to see how closely the rate of elongation of the growing asparagus stem conforms to the VAN'T HOFF-ARRHENIUS principle (10) the ratio of the rate of elongation at the end of various 10° C. (18° F.) intervals of temperature to that at the beginning has been calculated. According to this principle, the rate of growth may be expected to double for each 10 degrees of increase. It may be noted in table II that, over a considerable range of temperature, the rate of elongation of the stem does roughly double for an increase of 10° C. The rate almost exactly doubles as the temperature is raised from 16° to 26° C. for stalks of all heights except those 5 cm. tall. In the upper range of the temperatures prevailing in this study, the rate of elongation fails to double, while for intervals in the lower range it more than doubles for each rise of 10° C. It is apparent that this relationship between the prevailing temperature and the growth rate does not conform exactly to

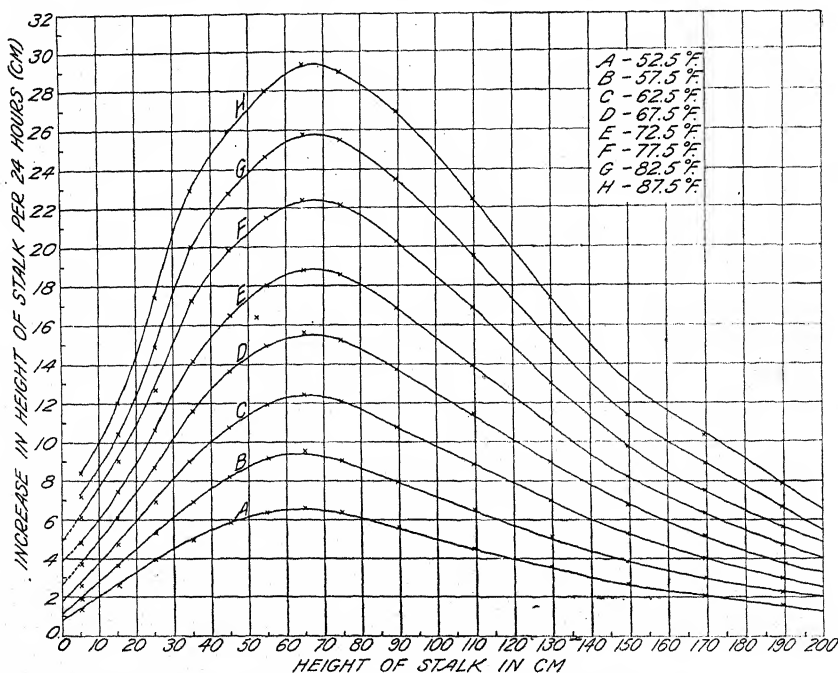


FIG. 3. Rate of growth of asparagus stalks at different heights for various temperatures expressed in cm. per 24 hours.

the VAN'T HOFF-ARRHENIUS principle. It may be inferred however, that it would be advantageous for a grower to cut the field twice as often at 26° C. (78.8° F.) as at 16° C. (60.8° F.).

#### RELATION OF HEIGHT OF STALK TO RATE OF GROWTH

Many writers have noted that the rate of growth varies continuously with differences in age or size of the plant. In the case of asparagus, WORKING (12) has presented the relationship very clearly. It may be noted from figures 1 and 2 that the stalks intermediate in height elongate much faster than the very tall or the very short ones. The relationship of the height of the plant to the rate of elongation is shown much more clearly in figure 3. As the material increases in height, the rate of elongation increases rapidly to a maximum and then gradually decreases to low values in the very tall stalks. The height of the stalk at which elongation is at a maximum is between 60 and 70 cm. for any given temperature.

Under the conditions of these measurements, the temperature might be low one day and high the next, or might vary in almost any manner. The present results are consequently a record of the response of plants of different heights to changes of temperature, and it should be remembered that the same magnitudes of change cannot be expected with material grown under uniform conditions of temperature throughout the entire period of their growth.

#### SUMMATION OF THE TEMPERATURE

It is clear from the above discussion that the height of the stalk at any time is a function of both time and temperature. It has often been considered that the development of the plant is dependent upon the amount of heat that it receives, the amount of heat being dependent upon the temperature and the length of the exposure. This idea has led to the working out of several systems of temperature indices (5, 7). One of the first of these is known as the remainder system, in which effective heat is estimated as total degree-hours above an arbitrary base temperature. The most essential thing in the employment of this system is the establishment of a correct temperature at which to begin counting the heat units. It has often been considered that the proper temperature for use as a base line is that at which growth first occurs. This may not always be the most practical or workable base line, as has been suggested by MAGOON and CULPEPPER (6). By extrapolation of the curves in figures 1 and 2 it may be inferred that growth in asparagus first becomes appreciable somewhere near 40° F., but no attempt was made to determine definitely the most appropriate base line. After considering the data to some extent it was concluded that 42.5° F. was fairly suitable, and using this as a base line, the temperatures have been summated for stalks 20, 50, 100 and 150 cm. in height, at 8 temperatures differing by 5° F. The results are shown in table III.



TABLE III

DEGREE-HOURS REQUIRED FOR ASPARAGUS STALKS TO GROW TO VARIOUS HEIGHTS WHEN EXPOSED TO VARIOUS ATMOSPHERIC TEMPERATURES, BEGINNING WITH STALKS 2.5 CM. BELOW THE SURFACE OF THE SOIL

HEIGHT OF STALK	GROWTH TEMPERATURE IN °F.							
	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5
	DEGREE-HOURS ABOVE 42.5° C.							
<i>cm.</i>								
20	3000	3312	3226	3000	2736	2688	2688	2700
50	4608	5076	4848	4860	4680	4512	4608	4536
100	6564	7164	7128	6900	6660	6510	6480	6480
150	9792	10476	10320	9960	9648	8324	9180	9660

It may be noted that roughly the same number of degree-hours are required for a stalk to grow from the base (2.5 cm. below the surface of the soil) to any given height for any temperature between 52.5° and 87.5° F. A perfect agreement could not be expected as the growth temperature relationship is only approximately linear. According to these calculations about 2900 degree-hours are required for a stalk to grow from the base to a height of 20 cm. and about 9800 degree-hours to grow to 150 cm. in height.

#### TIME REQUIRED FOR STALKS TO GROW TO DIFFERENT HEIGHTS

Knowing the rate of elongation of the stalk at all heights at every temperature, it is a simple matter to calculate the time required to reach any given height. By summing the growth rates day after day the number of days required to reach various heights have been obtained for material growing at various temperature levels. This was done by adding to the height of the plant at the end of the first day the growth made during the second day, which gives the height of the plant at the end of the second day, and so on until the ultimate height is reached. The data are given in table IV, and figure 4 shows very clearly the effect of temperature upon the time necessary to reach different heights. It may be expected that 6 days will be required for plants to reach a height of 100 cm. when the temperature averages 87.5° F.; 11.5 days when it averages 67.5° F.; and 27.2 days when it averages 52.5° F. The curves for 82.5 and 87.5° F. are of theoretical interest only for it is improbable that an average temperature as high as 87.5° F. for any 24-hour period would occur during the harvest season of asparagus. It must also be remembered that in this case the average daily temperature was never as high as 87.5° F. or as low as 52.5° F. for the entire growth period of any stalk and therefore these values will probably not hold for long periods of growth at the indicated temperatures. It is believed they will hold for short periods of growth and therefore may be of

TABLE IV

ESTIMATED HEIGHT OF STALKS OF ASPARAGUS AFTER GROWING FOR DIFFERENT LENGTHS OF TIME AT VARIOUS TEMPERATURES. ESTIMATED FROM THE GROWTH RATES AT DIFFERENT TEMPERATURES

GROWING PERIOD	GROWTH TEMPERATURE IN °F.							
	52.5	57.5	62.5	67.5	72.5	77.5	82.5	87.5
	HEIGHT OF STALK							
<i>Days</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>	<i>cm.</i>
0	0	0	0	0	0	0	0	0
2	1.5	2.2	3.6	5.5	8.1	10.8	13.2	15.7
4	3.4	5.3	8.9	14.0	20.8	29.0	36.7	47.2
6	5.9	9.4	16.7	27.2	42.5	63.0	82.3	102.7
8	9.1	15.3	28.0	48.5	76.9	106.3	127.2	145.0
10	13.2	23.3	45.0	78.2	111.4	138.0	155.5	171.0
12	18.5	34.0	67.6	106.4	136.6	159.4	175.4	190.2
14	25.1	48.3	91.7	128.6	155.1	175.7	191.0	204.8
16	33.3	66.1	111.7	145.8	169.7	188.9	203.3	
18	43.5	84.5	128.1	159.5	181.9	200.0		
20	55.2	100.5	141.7	170.9	192.2			
22	68.1	114.2	153.0	180.7	200.8			
24	81.0	126.0	162.7	189.2				
26	92.7	136.3	171.2	196.6				
28	103.2	145.4	178.8					
30	112.6	153.4	185.6					
32	121.1	160.6	191.8					
34	128.7	167.1	197.5					
36	135.7	173.2						
40	147.9	184.2						
44	158.3	193.6						
47	165.3	200.0						
48	167.5							
54	179.7							
60	190.0							
64	196.1							
67	200.3							

some practical importance in indicating how often the crop should be cut when the cutting of the stalks is to be made between certain definite heights.

According to these measurements, for stalks to grow from 10 cm. to 25 cm. in height, 5.3 days would be required at 52.5° F., 4.2 days at 57.5° F., 3.4 days at 62.5° F., 2.4 days at 67.5° F., 2.1 days at 72.5° F., and 1.9 days at 77.5° F. If a farmer restricted the cutting to stalks between these heights, he would have to cut the field at least as often as indicated or some of the stalks would be too tall at each cutting. In other words, it may be expected that about 1400 degree-hours of temperature would be required for stalks to elongate from 10 cm. to 25 cm. Of course the degree to which this rate of production would be realized would vary considerably with the fertility and moisture content of the soil, vigor of the plants, and possibly other factors. The plants in these tests were very vigorous as shown by the fact that the stalks finally reached a height of 230 to 250 cm.

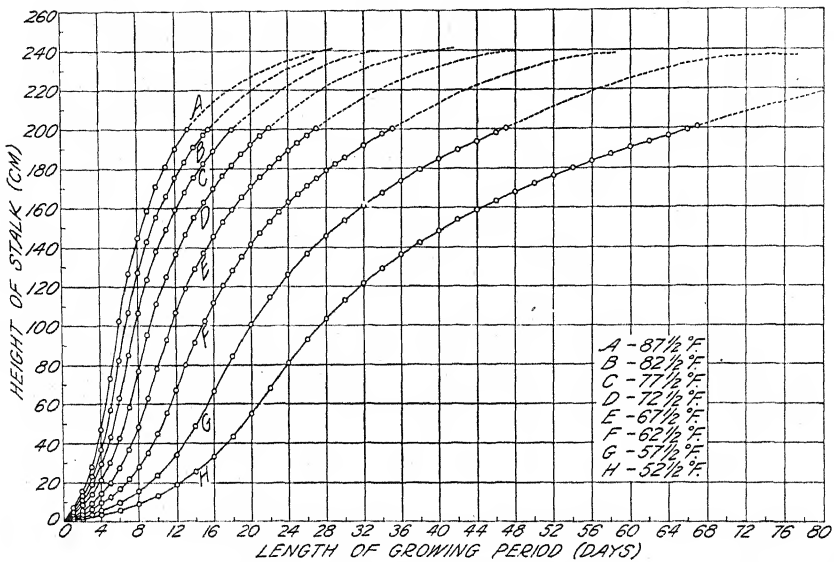


FIG. 4. Height in cm. of asparagus after growing for various lengths of time at various temperatures. The time values are expressed in days and the temperature in °F.

#### EFFECT OF TEMPERATURE UPON THE RATE OF ELONGATION ALONG THE STALK

The amount of elongation in different portions of the growing zone has been determined by WORKING (12). The rate of elongation per unit length of the stalk is maximum in a zone a short distance below the tip. The manner of growth is evident from figure 4.

It would be expected that the effect of temperature upon the rate of elongation in different zones along the stalk would be similar to the effect upon total elongation.

The results obtained from these measurements are given in table V and figure 5.

It is apparent from figure 5, L and M, that temperature affects the rate of growth of the stalk at every point throughout the entire growing region. The zone of maximum rate of growth, however, seems to be somewhat more sensitive to changes in temperature than zones above or below. The difference in sensitiveness is apparently small and may not be significant.

The curves indicate that growth stops at a point somewhat higher on the stalk at the low temperature than at the higher temperature. This may be attributed to error in selecting stalks for measurement.

The effect of the temperature upon the rate of growth at any point along the stalk appears to be approximately the same as for the total elongation, as shown in figures 1 and 2. In figure 5, N, the rates of growth at 5 different

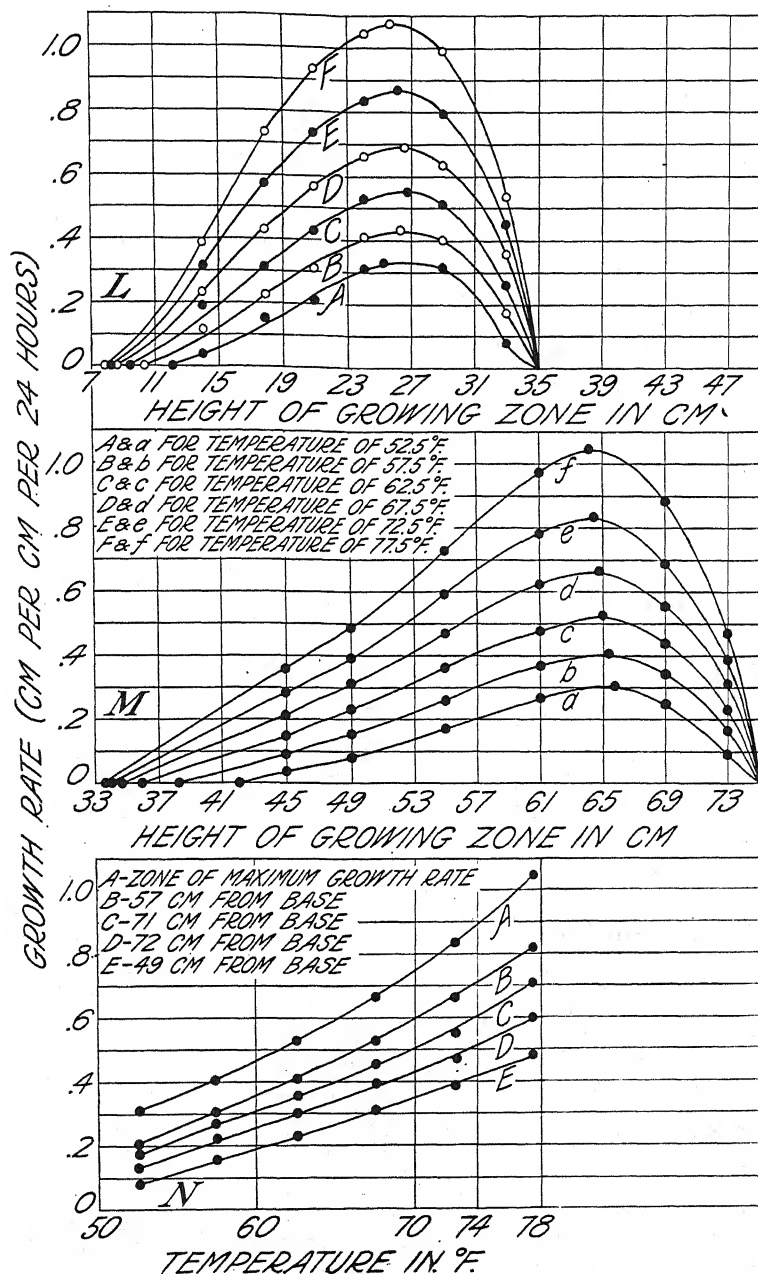


FIG. 5. Effect of temperature upon the rate of growth in various zones along the stalks of asparagus after they have reached the total height of 35 and 75 cm. L, stalks 35 cm.; M, stalks 75 cm. in height. N, the effect of temperature upon the rate of growth in different regions of stalks 75 cm. in height.

RATES OF GROWTH ALONG THE STALK OF ASPARAGUS UNDER VARIOUS TEMPERATURE CONDITIONS FOR STALKS 35 AND 75 CM. TALL, EXPRESSED IN CM. PER CM. PER 24 HOURS

[illegible]

locations along the stalk for material 75 cm. in height have been plotted against temperatures between 52.5° and 77.5° F. Each series of points forms loci that lie in almost a straight line. The slight curve slopes upward a little more sharply with the higher temperatures. It appears that the region of maximum growth is slightly closer to the base at high temperatures than at low temperatures. This may be a response that is related to the growth behavior early and late in the season. The differences are not large and may not be significant. These results should be interpreted as responses to variations in temperature during short intervals of time under field conditions and may not be the same as would occur under conditions where the temperature is constant. It has been shown by LEHENBAUER (3) and LEITCH (4) that the temperature for maximum growth depends upon the length of the period of exposure.

It may be concluded that the growth rate of asparagus is similar to that of many other plants and the results of these field measurements agree essentially with the results of SACHS (8) and many other investigators.

### Summary

1. The effect of temperature upon the rate of elongation of asparagus stalks has been studied under field conditions. Averages of a large number of measurements have been obtained by a method of cross classification, followed by a system of two-way plotting, the final values forming smooth curves when plotted in either of the two ways.

2. For temperatures between 52.5° and 87.5° F., the relationship between the growth rate and the temperature is represented by lines that are almost straight. The rate of total elongation approximately doubled with each increase of 10° C. (18° F.) over a limited range of temperatures.

3. The relationship between the rate of total elongation and the height of the stalk has been determined for the stalks in these tests and the results plotted. The rate of increase in height was slow at first, increased rapidly to about 65 cm. in height when it was at a maximum, and then slowly decreased as the stalks became taller.

4. The growth responses of these plants have been interpreted in terms of time required to reach different total heights. The lines representing this relationship take the form of the S-curve characteristic of growth processes. It may be seen from these rates that if it is desired to cut asparagus between the heights of 10 and 25 cm. the field would need to be cut at least every 5.3 days when the average temperature was 52.5° F., every 4.2 days when the average was 57.5°, every 3.4 days when it was 62.5°, every 2.4 days when it was 67.5°, and every 2.1 days when it was at 72.5° F. Likewise the time required for growth from any initial height to any other height at any designated temperature may be readily ascertained from the curves.

5. Variations in temperature affect the rate of growth along the stalk in about the same way as they affect the total elongation. The growth rate increases for a short distance below the tip to a point where it is maximum and then decreases to the lower limits of the growing region. The results indicate that the zone of maximum growth is a little more sensitive to changes of temperature than regions either above or below this zone.

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U. S. DEPARTMENT OF AGRICULTURE

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## PROBLEMS IN THE STORAGE OF CUT CARNATIONS

M. S. NEFF

(WITH TEN FIGURES)

Cut flowers are commonly stored with the stems in water. Such treatment results in a high turgor pressure within the plants which may be augmented by high humidity in the storage room. Preliminary experiments (3) indicated that the life of flowers is shortened by a liberal water supply during storage.

### Materials and methods

Comparisons were made between flowers stored in water and flowers stored "dry pack." When removed to ordinary temperature and humidity conditions, keeping qualities were observed and compared with fresh flowers. The flowers were arbitrarily classed as "desirable" until the edges of the petals first began to dry, or any other noticeable defect appeared. "Percentage desirable" as used in the following graphs indicates the number of "desirable" blooms expressed in percentage. The average number of flowers used in each lot was about nine.

"Dry pack" indicates storage without water. Flowers in "dry pack" were not placed in water until removed from storage. The methods of dry packing varied considerably. Wrappings of different kinds of paper were used in an attempt to find a suitable storage condition. A more successful method consisted of storing the flowers in sealed glass or metal containers. A refinement of this procedure made possible the control of the atmosphere under bell jars. Two-hole rubber stoppers supplied with glass tubing were fitted into the openings at the tops of the bell jars. Rubber tubing fitted to the outlets and closed by means of clamps facilitated gas analysis with the HALDANE gas analyzer. The outlets were also used for aeration and the introduction of carbon dioxide.

### Results

#### RELATION OF TURGOR TO THE KEEPING OF CUT CARNATIONS

The following experiments bring out marked differences in the behavior of carnations stored dry and those stored with the stems in water. Fluctuations in the weights of flowers were observed and the water loss per flower recorded during the period of observation at room temperature to determine the lasting qualities.

Carnations were placed in water at room temperature and humidity to determine their "desirable" length of life (fresh). Similar carnations were stored for 32 days with their stems in water. The stems were clipped



under water and the water changed 18 times during this period. (Water, 32 days.)

Carnations were also sealed in empty desiccating jars, one inverted over the other. The flowers were at no time placed in water until two hours before removal to room temperature. (Dry III, 32 days; Dry IV, 46 days.)

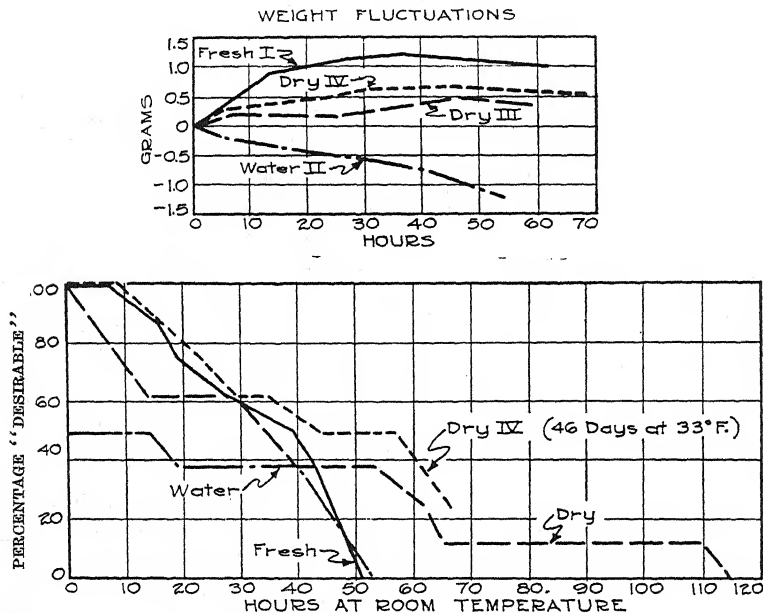


FIG. 1. "Wet" and "dry" storage carnations compared with fresh flowers at room temperature. Storage period, 32 days and 46 days at 33° F. Above, weight changes with time. Below, deterioration with time.

Carnations stored dry in sealed containers were in much better condition and lasted longer at room temperatures than carnations stored in water. On the average they stood up as well under room conditions as the freshly cut flowers.

Fresh carnations placed in water under room conditions gained in weight, reaching a maximum at the thirty-sixth hour. Carnations stored 46 days "dry pack," when removed and placed in water, underwent similar gains reaching a maximum at the forty-sixth hour. In contrast carnations stored for 32 days in water steadily lost weight at room temperature. The decrease in weight was strongly correlated with lack of keeping quality.

Flowers stored with the stems in water gained in weight and immediately had moisture available in unlimited quantities for any chemical or physical reactions. Although low temperatures greatly inhibit most plant processes, carnations stored in water tend to complete their developmental cycle. On

the other hand, "dry pack" flowers with judiciously lowered turgidity should have exceptional keeping qualities if moisture were a factor in hastening maturity.

Possibly more emphasis and importance should be attributed to the high carbon dioxide and low oxygen content within the sealed containers. Moderately high carbon dioxide has been found beneficial in flower storage (6).

It is interesting to note that the water loss at room temperature was approximately the same for fresh carnations, "dry pack" carnations, and those stored in water. This result might preclude the idea that increased life of the flowers at room temperature depended on higher transpiration.

The following determinations resemble the previous studies except no weighings were made. Fresh carnations were placed in water at room temperatures to determine the length of life. The stems of carnations stored in water were cut and the water changed several times during the storage period. The "dry pack" carnations were sealed in a dry glass jar and were at no time in water until two hours before removal to room temperature.

When carnations were placed at room temperature and humidity, the stems were cut and the water changed daily.

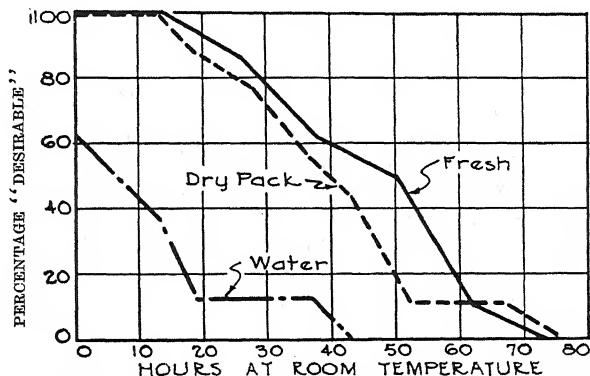


FIG. 2. "Wet" and "dry" storage carnations compared with fresh carnations at room temperature. Storage period, 37 days.

These results confirm the belief that turgor is an important factor in storage of carnations. "Dry pack" carnations compare favorably with fresh flowers as to keeping qualities at room temperature. Carnations stored with their stems in water were greatly inferior to fresh carnations.

In the following experiment additional differences in turgidity were produced by cutting the flowers in the heat of the day and in the evening, in addition to spreading the flowers out in the 40° F. room and wilting them for varying lengths of time in the relatively dry atmosphere of the room.

Fresh carnations were placed in water and "hardened" for 15 hours at

40° F. They were then placed at room temperature and humidity to determine the length of commercial life. Additional carnations were stored with the stems in water. Similar groups were precooled three and seven hours at 40° F. and sealed in dry glass containers. Because of the drying effect of the precooling room, the carnations "dry" and "dry delayed" were slightly wilted at the time of storage. The "dry pack" flowers were at no time placed in water until eight hours before removal to room temperature, when the stems were clipped, and the water was then changed every day.

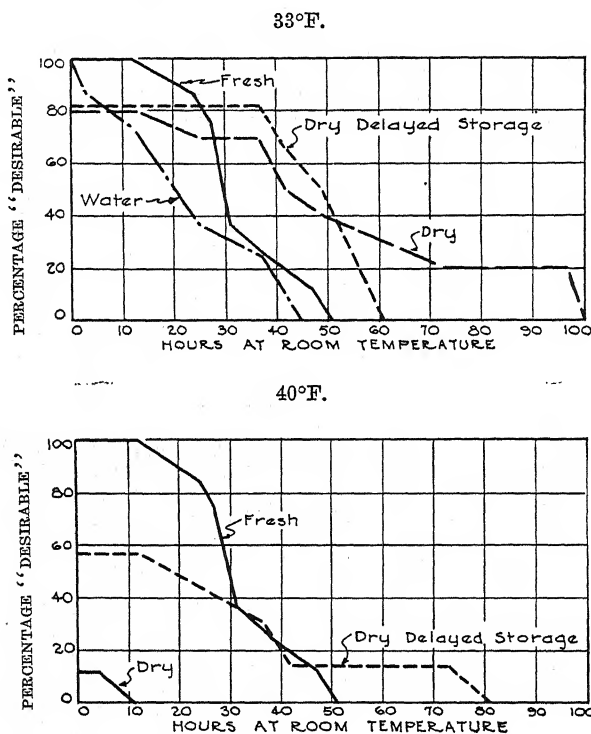


FIG. 3. "Wet" and "dry" storage carnations compared with fresh flowers at room temperature. Stored 39 days. Above, storage temperature 33° F. Below, storage temperature 40° F. "Dry delayed" were cut at noon and precooled for 7.5 hours. "Dry" were cut at 6 P. M. and precooled 3 hours. (Water stored flowers have been omitted from the 40° F. graph owing to their unsatisfactory condition.)

Carnations cut at midday and stored at 40° F. wilted slightly but were many times better than those picked in the evening, and were more turgid. It is probable that lowered turgidity dominated internal conditions and made possible the survival of the flowers. At 33° F. the differences were not so pronounced.

"Dry pack" carnations stored for 39 days lasted longer under room conditions than freshly picked flowers. This reaction is important, for the

moval from storage. As in previous experiments, the carnations stored dry lasted longer at room temperature than those stored in water.

The results also show the advisability of using low storage temperatures. At medium and high temperatures the respiration rate appeared to be too intense for long time storage of flowers. As the length of storage depends on minimizing respiration and other natural processes, the only alternative is the use of low temperatures.

The following graphs give additional evidence concerning water relations. Joan Marie carnations were shipped approximately 1100 miles and then placed in the low temperature room (33° F.), thus introducing a delay of 44 hours between cutting and the beginning of storage. Twelve hours later one group was sealed in a bell jar and a second lot was stored with the stems in water. The flowers in general were quite "tight," several being only one-half open at the time of storage.

Carbon dioxide was introduced into the storage bell jar to the extent of 6.4 per cent. The outlets were allowed to remain open and subsequent determinations showed that the gas percentage remained practically constant.

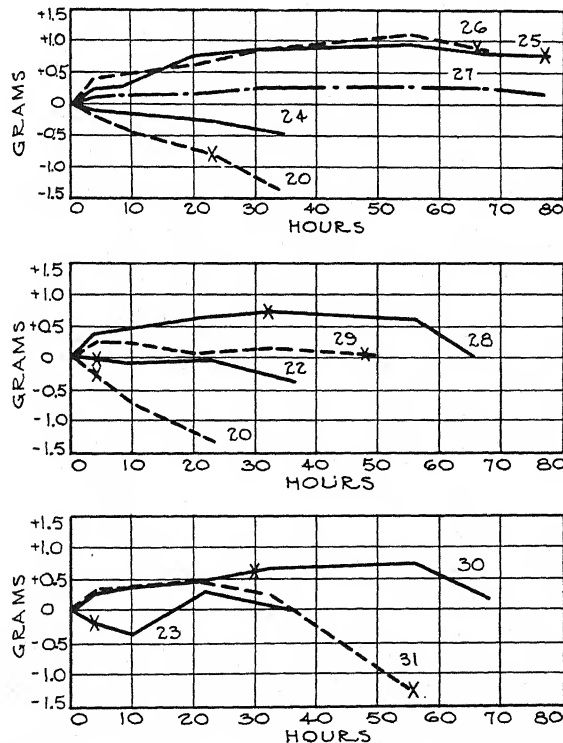


FIG. 4. Weight fluctuations of individual carnations at room temperature Nos. 20 to 24 were stored in water, while nos. 25 to 31 were stored dry. The flowers were stored 55 days at 33° F., following 44 hours delayed storage. Nos. 24 and 27 were not "desirable" at the time of removal. The position of X on each curve marks the close of the period of "desirable" condition.

Carnations stored "dry pack" were again superior to those stored in water. Previous results that the flowers showed increasing weight when placed in water were confirmed, while flowers stored in water lost weight when removed to room temperature.

There was a marked difference in the water loss at room temperature of flowers stored dry and those stored in water. It is probable that bacteria lodged in the conducting tissues in sufficient numbers to prevent the free absorption of water by the flowers stored in water (5). The water was changed only once during the 55 days of storage and as a result the stems were slimy and the water cloudy. It may be that after 55 days a combination of bacterial clogging and general breakdown occurred. A previous experiment, run for 32 days with only a few changes of water, resulted in a subsequent normal rate of water loss.

A preliminary carbohydrate analysis of the carnations shipped furnished additional information concerning turgidity. The analysis procedure was that described by LOOMIS and SHULL (2). All calculations were made on the extracted dry weight basis.

Sugars were determined from an 80 per cent. alcohol extraction (11 times). Dextrin and starch were determined from a hot water extract of the residue remaining from the alcohol extraction. The acid hydrolyzable fraction was obtained from the residue remaining from the dextrin-starch determinations.

The carbohydrates analyzed for were concentrated in the heads of the flowers. Considering heads alone, carnations stored 55 days "dry pack" were only slightly lower in total sugars than fresh carnations (table I). The same thing held true for glucose, fructose, sucrose, and reducing sugars. On the other hand, flowers stored with the stems in water were reduced in sugars by approximately one-half.

The dextrin and starch fractions of fresh and "dry pack" carnations were practically the same, while the same fractions in flowers stored in water were still considerably less. The more stable and less available acid-hydrolyzable fractions were practically the same in every case.

The analyses suggest a considerable utilization of available foods in the case of flowers stored in water, and a slight utilization when flowers were stored "dry pack."

Another group of carnations, shipped as before, were placed in the College low temperature room within 43 hours. Twelve hours later one group was sealed in a bell jar and a second lot placed in water at room temperature to determine the life of the fresh flowers. The flowers of the Joan Marie variety were tight when received; Pink Spectrum were fully open. The stored flowers were at no time in water until placed at room temperature. Carbon dioxide was held below 14 per cent. by frequent aerations.

TABLE I  
PRELIMINARY CARBOHYDRATE ANALYSIS OF JOAN MARIE CARNATIONS

FRACTIONS	No. I		No. II		No. III	
	FRESH CARNATIONS		STORED 55 DAYS STEMS IN WATER		STORED 55 DAYS "DRY PACK"	
	HEADS	STEMS	HEADS	STEMS	HEADS	STEMS
Green weight .....	37.85 gm.	26.60 gm.	52.62 gm.	25.6 gm.	45.8 gm.	21 gm.
Extracted dry weight .....	4.687 "	3.952 "	4.708 "	3.476 "	4.581 "	3.2527 "
Calculated as:						
Reducing sugars .....	32.49 %	1.97 %	16.52 %	0.00 %	29.54 %	0.00 %
Sucrose .....	5.57 "	1.88 "	3.35 "	2.46 "	5.65 "	1.21 "
Fructose .....	7.08 "	0.00 "	3.56 "	0.00 "	6.48 "	0.00 "
Glucose .....	25.41 "	1.97 "	12.96 "	0.00 "	23.06 "	2.32 "
Total sugars .....	38.06 "	3.85 "	19.83 "	2.46 "	35.19 "	1.21 "
Dextrin and starch .....	4.90 "	2.11 "	3.84 "	1.98 "	4.86 "	2.25 "
Acid hydrolyzable .....	14.28 "	8.50 "	13.14 "	8.35 "	13.87 "	8.52 "
Total carbohydrates .....	57.24 "	14.46 "	35.84 "	12.79 "	53.92 "	11.98 "

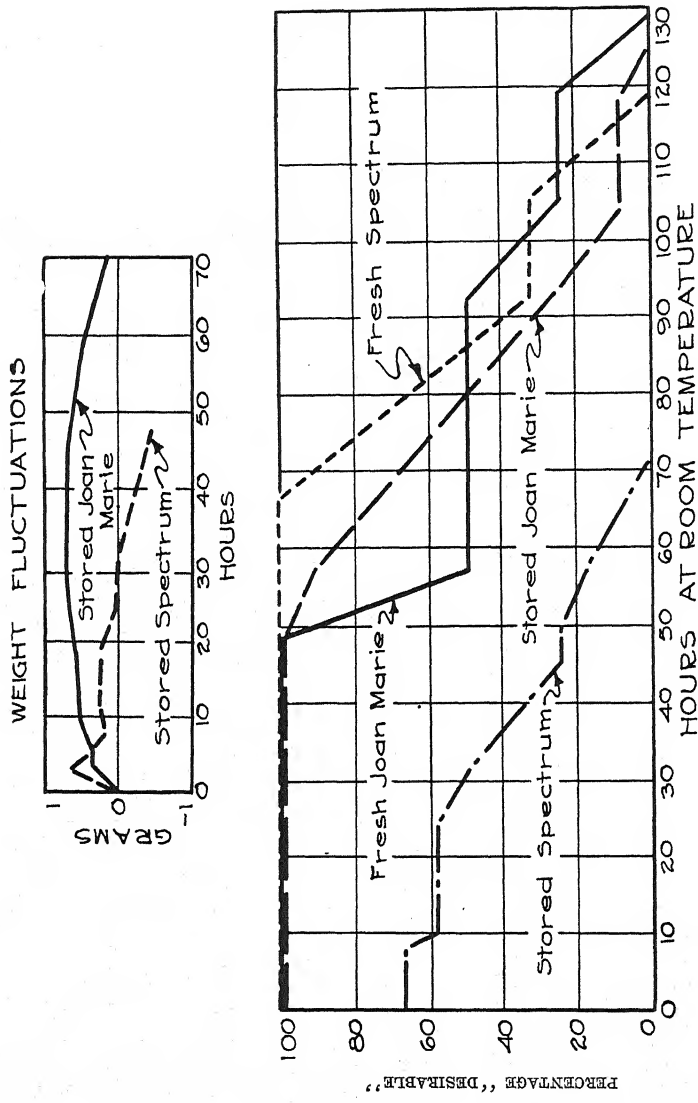


Fig. 5. Carnations stored for 56 days at 33° F. compared with fresh flowers at room temperature. Above, weight changes with time. Below, changes in condition with time.

Fresh Pink Spectrum flowers were superior to Joan Marie, but were much inferior after storage. Pink Spectrum flowers were considerably more advanced in maturity than Joan Marie at the time of storage.

Both kinds of carnations gained in weight in water at room temperature. Joan Marie made a moderate initial gain and steadily gained in weight for 31 hours. This is the normal reaction of fresh carnations. Pink Spectrum reached its maximum in the first few hours and gradually decreased in weight. Except for the first large gain, the Pink Spectrum carnations approximated closely the typical weight losses of flowers stored in water. Their "desirable" length of life at room temperature also paralleled the results with water-stored flowers. The high water absorption in the first few hours accounts for the increased weight of the flowers. More water goes into the hydration of tissues than is transpired.

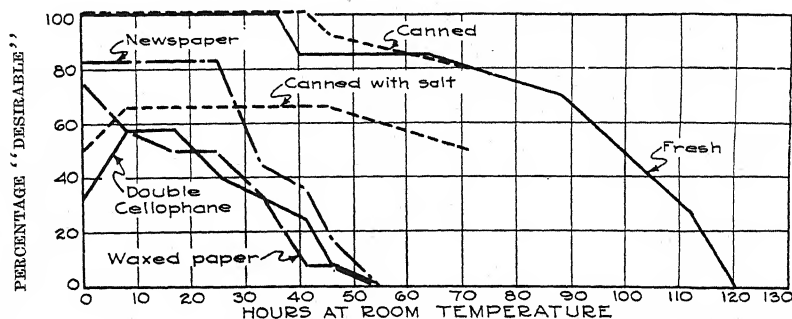


FIG. 6. Results from different methods of "dry pack" storage of carnations. Storage period, 27 days at 34° F.

#### RELATIONS OF HUMIDITY TO THE KEEPING OF CUT CARNATIONS

HITCHCOCK and ZIMMERMAN (1) found that cut carnations did best in a relative humidity above 98 per cent. The storage of carnations in sealed containers is ideal from the point of view of humidity for in a few hours moisture condenses inside, thus indicating a saturated atmosphere.

Carnations were cut and stored at 34° F. within 2.5 hours after cutting. Lots of flowers were wrapped in newspaper, in waxed tissue paper, and in cellophane; gallon honey cans with tight fitting lids were also used. The flowers were at no time placed in water until removed to room temperature and humidity. The stems were then clipped and the water was changed every day.

The flowers stored in sealed cans kept more successfully (fig. 6) than those wrapped in newspaper, waxed paper (fig. 9, IV), or cellophane (fig. 9, V). The carnations in the can without salt were fully open (fig. 7, VI) and in excellent condition. They compared very favorably with fresh car-



nations when placed at room temperature and humidity. Some of the carnations stored in the can containing salt (fig. 7, III) were badly decomposed; the others were in fair condition. Two additional blossoms became usable after a few hours in water. The object of the salt was to reduce the humidity but both cans contained moisture, indicating a saturated atmosphere. Excessive desiccation injured the wrapped flowers, those in newspaper (fig. 7, II) being the least affected.

It is interesting to compare the carnations stored in a can (fig. 7, VI, and fig. 10) with those stored by other means. Flowers stored in the sealed gallon can were fully open, crisp, and had firm calyces; the others were soft and small, as shown in figure 7. After 22.5 hours in water at room temperature the flowers stored in the gallon can were still superior (fig. 8, IV); those remaining from the can with added salt were equal in quality to



FIG. 7. Condition of carnations at the time of removal (storage period 27 days). II, stored wrapped in newspaper. III, stored in a gallon can with salt. V, stored wrapped in double cellophane. VI, stored in a gallon without salt.

those from the first can. The carnations which had been wrapped and stored in newspapers (fig. 8, II) still lacked the size and firmness of those stored in cans.

### Discussion

The successful storage of cut carnations depends upon arresting the normal development of the flowers without initiating undesirable physiological reactions. It is not only necessary that the flowers should look well when removed from storage, but they must hold up satisfactorily at room

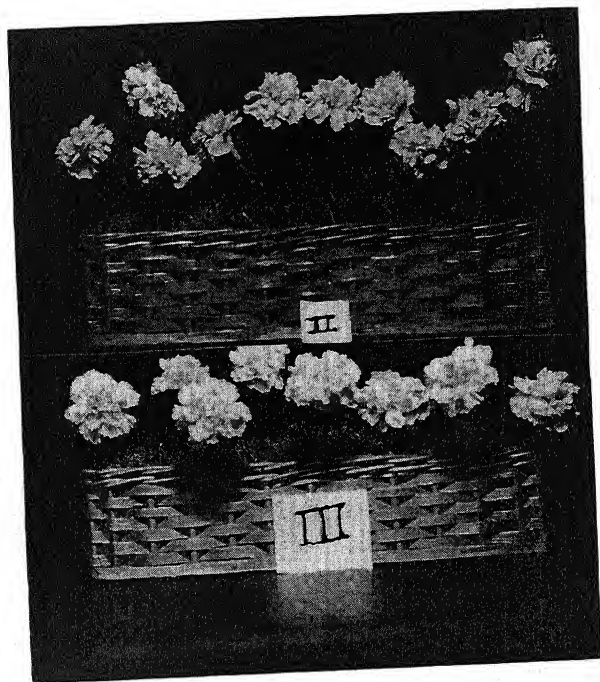


FIG. 8. Condition of carnations after 22.5 hours at room temperature. Only flowers of "desirable" grade were photographed. II, stored wrapped in newspaper. 83 per cent. were "desirable." III, stored in a gallon can with salt. 70 per cent. were "desirable."

temperature. Low temperature is the storage factor upon which most dependence is placed. It has been pointed out, however, that carnations are unfavorably affected by the usual storage at low temperatures. Other variables which may enter into the problem are: the turgidity of the flowers during storage; the humidity of the storage atmosphere and its gas content, particularly the percentage of carbon dioxide and oxygen; and finally the condition and inherent storage qualities of the flowers it is desired to hold. The importance of low temperatures for long time storage is self-evident.

Both respiration and development toward maturity must be kept at a minimum if the flowers are to survive.

In addition to the use of low temperatures to slow respiration it is probable that more attention should be given to the reserves of the flowers at the time of storage.

The importance of reserves is evidenced by the greatly reduced carbohydrate percentage in flowers stored in water and their corresponding lack in keeping qualities. "Dry pack" carnations, on the other hand, were com-

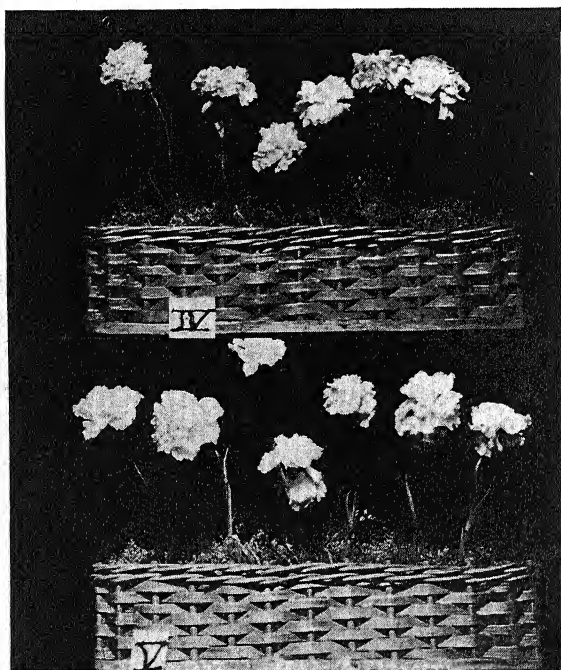


FIG. 9. IV, stored wrapped in waxed paper. 50 per cent. were "desirable" (border-line case). V, stored wrapped in double cellophane. 58 per cent. were "desirable."

parable with fresh flowers as to keeping qualities and food reserves. Pre-picking treatments, such as harvesting on the afternoon of a sunny day, and watering lightly for a day or two beforehand, should theoretically increase the storage period.

Turgor as well as temperature has an important rôle in growth and maturity. The experiments show that unlimited moisture during the storage period is generally detrimental to the keeping qualities of cut carnations. PRIESTLEY (4) states that division and elongation of the cells in a growing meristem depend on high moisture content at the growing point. It is not

unexpected, therefore, to find that partly matured carnations opened fully during storage in water, while comparable flowers, dry packed in sealed containers, developed much more slowly and opened very little during the storage period. NEFF and LOOMIS (3) concluded that maturity and old age is the result of cell development. Cell development can progress normally only when there is sufficient moisture present to maintain high turgor pressure. Reducing the turgor pressure, therefore, reduces the rate of maturity. Controlled wilting previous to storage by the "dry pack" method shows considerable promise.

Wrapping flowers in various materials can probably be used to control humidity around the flowers and prevent excessive drying. The correct

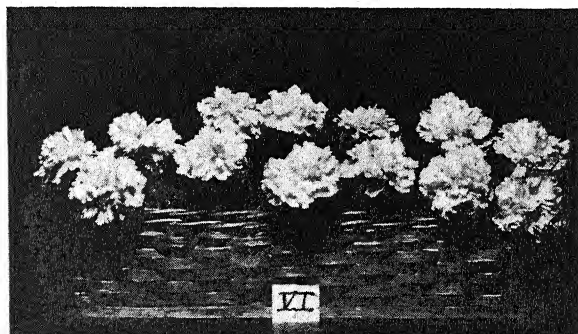


FIG. 10. VI, stored in a gallon can. 100 per cent. were "desirable."

material and number of wrappings could be determined only by trial, and a standardization applicable to all storage rooms and flowers would be impossible. The use of sealed containers reduces the guess work. A few hours after being placed in low temperature, moisture collects on the sides of containers, indicating a saturated atmosphere.

The degree of tightness of the container influences the accumulation of carbon dioxide and reduction of oxygen. For long storage periods an accurate knowledge of the carbon dioxide and oxygen percentages is necessary, otherwise injury, or lack of injury, is a matter of luck. Unquestionably, the benefits derived from carbon dioxide accumulation and oxygen decrease in many of the experiments were offset by the injurious effects of high carbon dioxide and low oxygen present at the end of the experiments. The best results were obtained when the containers were aerated.

The poor keeping qualities of carnations stored in water could not be attributed to lack of ability to absorb water. In only one experiment was there a reduction in rate of water loss from "wet pack" as compared to fresh, or "dry pack" flowers.

### Summary

1. Carnations stored at 33° F. were superior to those stored at 40° F.
2. Carnations stored without being placed in water, were comparable, in keeping quality and carbohydrate reserves, with fresh carnations.
3. Carnations stored with the stems in water were inferior in keeping quality, and low in carbohydrates.
4. Fresh and "dry pack" storage carnations increased in weight when placed in water at room temperature. Carnations stored in water lost weight at room temperature. There was a correlation between weight fluctuations and keeping quality.
5. Carnations wilted previous to storing "dry pack" were superior to more turgid "dry pack" carnations.
6. In general, fresh "dry pack" and water stored carnations had the same rate of transpiration.
7. Carnations were favored by a high humidity. Sealed containers produced this condition; wrapped carnations suffered from desiccation.

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# APPLICATION OF THE CERIC SULPHATE METHOD IN THE ANALYSIS OF CARBOHYDRATES IN THE ROOTS OF LEPIDIUM AND CONVULVULUS<sup>1</sup>

C. G. BARR

(WITH FOUR FIGURES)

## Introduction

During the course of a general investigation of the carbohydrates in the roots of perennial weeds it was found that copper reduction methods commonly employed for sugar determinations were in some respects unsatisfactory for the analysis of whiteweed, *Lepidium*. The greatest difficulty was encountered in the determination of the total sugar content from the alcoholic root extract. This experience led to further investigation to discover suitable methods for this work.

The ceric sulphate method adopted is one which has been described (2) and shown to be useful in the determination of varying quantities of reducing substances in plant tissue. The writer found it to be workable for estimating moderately large quantities of sugars, especially in the determination of total sugars in *Lepidium* by invertase hydrolysis. It has the distinct advantage in being useful in experiments involving the analysis of great numbers of samples. The use of ceric sulphate eliminates the steps of precipitating, filtering and measuring reduced copper which were the sources of difficulty encountered in the analysis of the soluble carbohydrates in *Lepidium*.

## Materials and methods

The material used was the roots of *Lepidium draba* var. *repens*, growing in the vicinity of Fort Collins, Colorado. The samples used for the experiments on methods were aliquots from the extracts and portions of the residues of those samples which had been collected for a study of the reserve carbohydrates.

The fresh roots were killed in boiling 95 per cent. alcohol, the soluble carbohydrates extracted by a series of decantations with 80 per cent. alcohol, and the total made to a convenient volume. Aliquot portions of the extract were taken for analysis, the alcohol removed, and the resulting water solutions cleared with neutral lead acetate and made to volume. Deleading was accomplished by filtering into beakers containing anhydrous sodium oxalate. Sodium oxalate, however, cannot be used as a deleading agent when the

<sup>1</sup> Contribution from the Colorado Agricultural Experiment Station in cooperation with the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Published with the approval of the Director of the Experiment Station.

analyses are made with ceric sulphate since the oxalate is oxidized by ceric sulphate. Dry dipotassium phosphate is therefore recommended instead of the sodium oxalate.

No difficulty was encountered when the cleared solution was boiled with Fehling's solution for reducing sugar determinations. Only small quantities of reducing sugars, however, were found to be present. For total sugar determinations 50-ml. samples of the cleared solution were adjusted to the proper pH with 10 per cent. acetic acid and about 6 drops of 1 per cent. solution of invertase scales were added. Extracts containing sucrose are hydrolyzed to invert sugars in about two hours at 20° C. The extracts of bindweed and most other plant tissues are satisfactorily analyzed in this manner for total sugars by the MUNSON-WALKER (4) method of copper reduction but in the analysis of *Lepidium* considerable difficulty was encountered.

Upon heating the hydrolyzed samples of *Lepidium* with Fehling's solution, the contents of the beaker first appeared dark, then passed through several shades of bluish green and finally became reddish brown by the time the solution boiled. After boiling for two minutes and filtering off the excess Fehling's solution, the precipitate remaining in the Gooch filter was rather dark brown in color and not completely soluble in the ferric ammonium sulphate solution used in the modified BERTRAND (1) method for the estimation of copper. Considerable residue always remained in the filter even after breaking up the asbestos mat and treating a number of times with the alum solution. Samples were diluted and tests were run which showed that the peculiar behavior was not due to a high concentration of the sugar, although dilution to some extent reduced the difficulty in filtering the cuprous oxide. Fluctuating values were obtained with potassium permanganate titrations on duplicate samples and frequently different values were obtained from the same cleared and deleaded sample. These results suggested the possibility that some substance might have been left in the samples by the clearing reagent which interfered with copper reduction. Samples were therefore cleared with Horne's dry subacetate, but these samples showed the same reaction. This behavior indicated that the material causing the difficulty in the analysis was probably carbohydrate in nature.

When hydrochloric or sulphuric acid was used for hydrolysis instead of the enzyme, hydrolysis appeared to be complete and the substances contained in the hydrolysate reacted with the Fehling's solution to produce a cuprous oxide precipitate which behaved more normally.

No attempt is made in this paper to present evidence for the identification of the material present causing the difficulty, the object being to show that for sugar analysis of *Lepidium* a shorter and more convenient method may be used which yields comparable and trustworthy results.

The expense of chemicals and the labor involved in the analysis of plant

tissue by the several copper methods are items of primary importance in cases where funds are limited. This is especially true if the investigation requires the analysis of large numbers of samples for the various carbohydrate fractions.

HASSID (2) has shown that ceric sulphate may be used in the determination of varying quantities of sugar in plant tissue. He presented data on five different dry plant materials, and showed that the results by this method were as much as 5 per cent. higher than by the MUNSON-WALKER method.

Preliminary tests of the two methods were made by the writer on leaves of maize and bindweed and also on the roots of bindweed and white weed. Reducing sugar and total sugar values were from 4 to 10 per cent. higher when determined by the ceric sulphate method than by the modified MUNSON-WALKER method. Apparently the variation was dependent upon the tissue under investigation. The results showed closer agreement between the two methods for vegetative tissue than for root material.

As described by HASSID, the principle involves the reduction of alkaline potassium ferricyanide by the reducing substances in the sample and the titration of the reduced iron with a standard ceric sulphate solution. The ceric sulphate is standardized against 0.1 N ferrous sulphate and the glucose factor determined by checking with a carefully prepared 0.1 per cent. solution of pure dextrose.

The solutions used are prepared as follows:

A. Alkaline potassium ferricyanide

4 gm. of potassium ferricyanide and 24 gm. of sodium carbonate are dissolved in distilled water and made up to one liter. This solution is quite stable if kept in the dark in a brown bottle.

B. Ceric sulphate

8.3 gm. of c. p. ceric sulphate are dissolved in about 50 ml. of distilled water in a one liter volumetric flask. To this is added 100 ml. of 1:1 sulphuric acid and made to volume.

To 10 ml. aliquots of the sugar solution in 200-ml. Erlenmeyer flasks, 25 ml. of the alkaline potassium ferricyanide are added. This is heated on a boiling water bath for exactly ten minutes and then cooled in running tap water. The solution is then neutralized with 10 ml. of 5 N  $\text{H}_2\text{SO}_4$  and titrated with the standard ceric sulphate using about 3 drops of 0.025 M ortho-phenanthroline ferrous sulphate complex as an inside indicator. It was found that the indicator solution may be diluted with distilled water to 50 per cent. of the above concentration and used with satisfactory results. HASSID (3) has also shown that Setopaline C is a more suitable indicator than ortho-phenanthroline<sup>2</sup> for the determination of quantities of sugar from 0.3 to 3.50 mg. per sample.

<sup>2</sup> The ceric sulphate and ortho-phenanthroline may be obtained from the G. Frederick Smith Chemical Company, Columbus, Ohio.



A simple calculation gives the sugar content in percentage of green weight. If 0.011626 N ceric sulphate is used, standardizations and tests

TABLE I

CARBOHYDRATES\* OF THE WHITE WEED GROWN ON UNCULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON- WALKER METHOD		CERIC SULPHATE METHOD	
	TOTAL SUGARS	TOTAL CARBOHYDRATES	TOTAL SUGARS	TOTAL CARBOHYDRATES
	%	%	%	%
April 25				
(1-2) .....	0.55	4.66	1.50	6.54
(4-5) .....	1.35	6.65	2.82	6.59
May 9				
(18-19) .....	0.96	6.26	1.77	7.74
(20-21) .....	1.51	6.60	2.26	7.76
May 25				
(55-56) .....	1.25	11.55	2.15	13.08
(57-58) .....	1.64	11.69	2.88	12.39
June 8				
(71-72) .....	1.68	15.55	2.77	24.75
(73-74) .....	2.30	14.16	3.95	23.39
June 22				
(79-80) .....	0.95	17.20	1.91	23.18
(81-82) .....	1.81	16.50	3.00	22.54
July 6				
(95-96) .....	1.41	21.20	3.76	30.24
(97-98) .....	2.40	20.00	4.16	27.74
July 21				
(102-103) .....	1.68	23.12	2.96	31.25
(104-105) .....	2.40	21.28	3.86	29.30
August 2				
(125-126) .....	1.54	22.24	2.77	28.75
(127-128) .....	1.70	22.59	2.88	29.64
August 18				
(147-148) .....		24.00	2.20	24.25
(149-150) .....	0.88	22.10	2.99	26.50
Sept. 1				
(159-160) .....	0.94	21.52	1.98	24.95
(161-162) .....	1.68	21.94	2.96	26.34
Sept. 13				
(177-178) .....	1.23	19.70	2.16	22.53
(179-180) .....	1.35	20.94	2.24	26.64
Sept. 30				
(189-190) .....	1.72	18.52	2.51	23.57
(191-192) .....	1.40	20.08	2.04	26.28
Oct. 16				
(201-202) .....	2.67	17.10	3.49	22.89
(203-204) .....	2.23	19.60	3.10	24.79
Oct. 29				
(209-210) .....	4.31	17.40	5.46	25.46
(211-212) .....	3.19	18.29	4.36	27.05
Dec. 3				
(217-218) .....	6.34	14.30	7.44	20.22
(219-220) .....	4.52	15.40	6.65	23.72

\* Expressed as percentage of green weight.

against pure glucose show that 2.47 ml. are equivalent to 1 mg. of glucose. One hundred ml. of extract cleared and made to 250 ml. volume represents one-fifth of the original alcoholic extract which contains the total amount of sugars from 50 gm. of fresh tissue. If a 10-ml. aliquot of this cleared solution is used in the sugar determination and a titration of 14.28 ml. of ceric sulphate is obtained after deducting the reagent blank, the per cent. sugar would be calculated as follows:

$$\left(\frac{500}{100}\right) \times \left(\frac{250}{10}\right) \times \left(\frac{14.28}{2.47}\right) \times \left(\frac{100}{50000}\right) = 1.44 \text{ per cent.}$$

Solutions containing large quantities of reducing sugars may contain more than 20 mg. of glucose equivalent to the 10 ml. aliquots, in which case the 25 ml. of ferricyanide will not contain sufficient ferric iron. This may be avoided in two ways: (1) by proper dilution of the sugar solution or (2) by making up the ferricyanide solution to contain more iron. The latter method was used in this investigation since there is probably less error involved in this procedure than in further dilution of the sample. When higher sugar values are to be determined the concentration of the ceric sulphate should be correspondingly increased to 0.02 to 0.03 N in order to obtain suitable titration values.

TABLE II

CARBOHYDRATES\* OF THE WHITE WEED GROWN ON CULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON- WALKER METHOD		CERIC SULPHATE METHOD	
	TOTAL SUGARS	TOTAL CARBOHYDRATES	TOTAL SUGARS	TOTAL CARBOHYDRATES
	%	%	%	%
May 9				
(22-23) .....	0.39	5.40	1.38	5.74
(24-25) .....	1.70	5.50	2.79	8.47
May 25				
(51-52) .....	0.14	4.03	1.10	6.70
(53-54) .....	1.30	5.53	2.68	8.77
June 8				
(67-68) .....	0.22	3.40	1.00	8.84
(69-70) .....	0.89	3.90	1.60	7.24
June 22				
(75-76) .....	0.22	3.50	1.12	5.79
(77-78) .....	0.10	3.40	1.01	6.02
July 6				
(91-92) .....	0.30	3.60	1.42	7.20
(93-94) .....	0.40	4.30	1.85	7.52
July 21				
(99- ) .....	0.50	5.40	2.11	9.25
(101-102) .....	0.50	5.20	2.12	9.08
August 2				
(122- ) .....	0.70	4.36	2.52	8.10
(123-124) .....	1.98	6.50	3.17	8.33

\* Expressed as percentage of green weight.

Samples of *Lepidium* which had been collected for a study of the trend of root carbohydrates at two-week intervals from April 25 to October 29, 1936, were used also for this study. Data are presented in table I comparing the total sugar values in percentage of green weight as determined by the MUNSON-WALKER method of copper precipitation and by the ceric sulphate method. Each value represents the average of duplicate samples. Tests were made on root samples from the first- and second-foot levels from plants growing on cultivated and undisturbed plots. A total of 220 separate white weed samples were analyzed for sugar content by these two methods. A study of tables I and II shows that the general trend of total sugar is the same when estimated by either of the two methods. The variations are of the same order but somewhat more distinct when calculated by the ceric sulphate method. The higher values and greater variations may be accounted for by assuming that the sample contains certain substances and impurities, such as acetates or citrates, which have a reducing effect upon the ferri-cyanide.

The total sugar values obtained by the two methods for the samples from uncultivated plots are shown graphically and compared in figure 1.

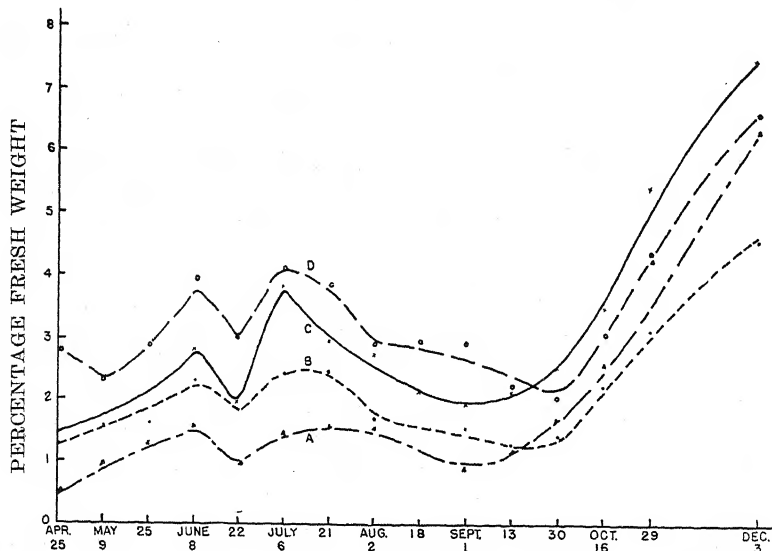


FIG. 1. Comparison of the ceric sulphate and MUNSON-WALKER methods for total sugars in white weed roots. A—First foot level by the MUNSON-WALKER method. B—Second foot level by the MUNSON-WALKER method. C—First foot level by the ceric sulphate method. D—Second foot level by the ceric sulphate method.

It is observed that the curves representing the percentages by the two methods are nearly parallel throughout.

Data are given in tables III and IV for *Convolvulus arvensis* which show

TABLE III

SUGARS\* OF THE BINDWEED GROWN ON UNCULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD			CERIC SULPHATE METHOD		
	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE
	%	%	%	%	%	%
April 25 (1-2) .....	0.55	2.44	1.91	1.24	3.11	1.87
May 9 (11-12) .....	0.51	1.91	1.40	0.83	2.24	1.41
May 25 (21-22) .....	0.66	2.25	1.59	1.01	2.52	1.51
June 8 (31-32) .....	1.07	2.54	1.47	3.90	6.14	4.24
June 22 (35-36) .....	0.83	2.81	1.98	3.01	4.93	1.91
July 6 (43-44) .....	0.77	2.66	1.89	1.05	3.03	1.98
July 20 (55-56) .....	1.31	3.94	2.63	2.04	4.52	2.48
August 1 (63-64) .....	1.21	3.96	2.75	2.08	4.90	2.82
August 17 (73-74) .....	1.25	2.79	1.54	2.04	3.46	1.42
August 31 (83-84) .....	1.56	3.97	2.41	2.32	4.77	2.45
Sept. 16 (93-94) .....	1.62	4.46	2.84	2.13	5.19	3.06
Sept. 29 (99-100) .....	1.92	5.77	3.85	2.90	6.94	4.04
Oct. 15 (123-124) .....	1.67	5.83	4.16	2.05	6.95	4.90
Oct. 30 (127-128) .....	1.00	7.09	6.09	1.70	7.77	6.07

\* Expressed as percentage of green weight.

somewhat closer agreement by the two methods on the total sugar values for this material than for white weed. The curves in figure 2 are so nearly parallel that the trends are identical.

Bindweed roots contain appreciable quantities of reducing sugars and the reducing values obtained by each of the two methods exhibit the same fluctuations. It was mentioned in an earlier part of this paper that bindweed roots behaved normally toward invertase when analyzed for total sugars by the MUNSON-WALKER method of precipitation. Tables III and IV show the sucrose values for bindweed roots by the MUNSON-WALKER and ceric sulphate methods respectively. Comparison of these values shows strikingly close agreement for sucrose by the two methods. There are but two samples in the series which show greater than 0.2 per cent. variation in sucrose by the two methods of analysis. Several additional tests were

TABLE IV

SUGARS\* OF BINDWEED GROWN ON CULTIVATED PLOTS, 1936

DATE AND SAMPLE NUMBER	MODIFIED MUNSON-WALKER METHOD			CERIC SULPHATE METHOD		
	REDUCING SUGARS	TOTAL SUGARS	SUCROSE	REDUCING SUGARS	TOTAL SUGARS	SUCROSE
	%	%	%	%	%	%
May 25 (23-24) .....	0.53	1.50	0.97	0.91	2.12	1.21
June 8 (29-30) .....	0.40	1.77	1.37	2.00	5.38	3.38
June 22 (33-34) .....	0.26	1.19	0.93	1.66	4.15	2.49
July 6 (45-46) .....	0.03	0.82	0.79	0.61	1.18	0.57
July 20 (53-54) .....	0.01	0.70	0.69	0.41	0.85	0.44
Aug. 1 (61-62) .....	0.02	0.71	0.69	0.77	1.30	0.53
Aug. 17 (71-72) .....	.....	0.71	0.71	0.81	1.30	0.49
Aug. 31 (81-82) .....	0.20	0.98	0.78	0.46	1.13	0.61
Sept. 16 (91-92) .....	0.17	0.54	0.37	0.46	0.84	0.38
Sept. 29 (101-102) .....	0.11	0.80	0.69	0.47	1.08	0.61
Oct. 15 (121-122) .....	.....	0.95	0.95	0.46	1.55	1.09

\* Expressed as percentage of green weight.

made on the extract of these samples but each time the results were identical.

By referring to figure 2 it will be observed that approximately the same difference exists between the two methods of analysis for either reducing sugars or total sugars. It has been shown above that calculating the percentage of sucrose as the difference between the total sugars and the reducing sugars yields the same values by either method. Since the same error is present whether analyses are made for total or reducing substances the trend, of course, remains the same. The question arises, however, whether the higher values obtained for reducing substances by the ceric sulphate method actually represent the reducing sugar value or whether the apparent higher sugar value is due to the reducing action of other substances contained in the sample such as citrate and acetate buffers. WHITMOYER (5) reports that chlorides and tartrates have practically no effect upon the reduction of ferricyanide by invert sugar while acetates and citrates slightly affect the reduction.

In order to determine whether or not the higher values were the result of reducing action by substances other than sugars, which could be removed

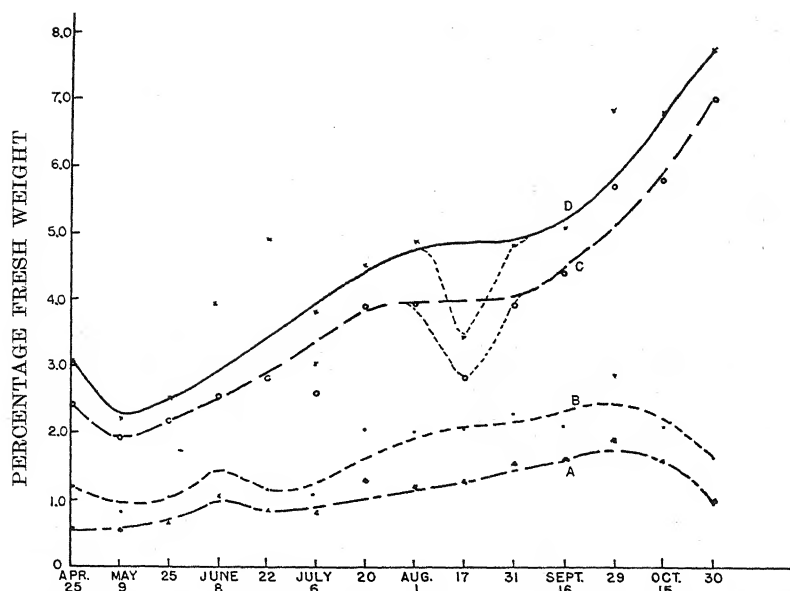


FIG 2. Comparison of the ceric sulphate and MUNSON-WALKER methods for reducing and total sugars in bindweed roots. A—Reducing sugars by the MUNSON-WALKER method. B—Reducing sugars by the ceric sulphate method. C—Total sugars by the MUNSON-WALKER method. D—Total sugars by the ceric sulphate method.

by activated carbon, tests were made on samples treated with carbon in comparison with untreated samples. Data presented in table V give the results

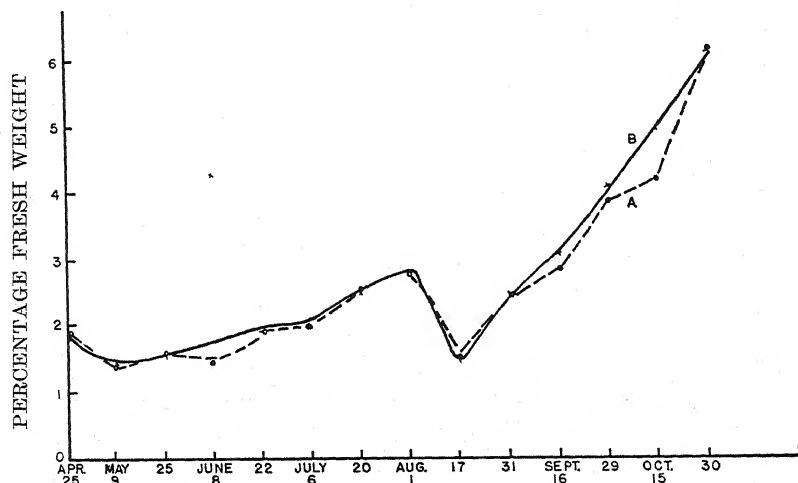


FIG 3. Comparison of the ceric sulphate and MUNSON-WALKER methods for sucrose in bindweed roots. A—Sucrose by the MUNSON-WALKER method. B—Sucrose by the ceric sulphate method.

TABLE V

EFFECT OF VEGETABLE CARBON "NUCHAR" UPON ANALYSES OF PLANT SUGARS  
AND PURE SUCROSE

SAMPLE NO CARBON	ANALYSIS BY MUNSON-WALKER METHOD				ANALYSIS BY CERIC SULPHATE METHOD			
	TITRA- TION	NET AVE.	COPPER	DEX- TROSE	TITRA- TION	NET AVE.	DEX- TROSE	DEXTROSE PER 50 CC.
	cc.	cc.	mg.	mg.	cc.	cc.	mg.	mg.
97 .....	16.80	16.20	102.70	50.20	18.70	18.05	12.45	62.25
98 .....	16.40	.....	.....	.....	18.20	.....	.....	.....
99 .....	18.60	18.00	114.10	56.10	20.00	19.95	13.75	68.75
100 .....	18.20	.....	.....	.....	20.70	.....	.....	.....
107 .....	5.90	5.50	34.90	16.60	6.40	6.00	4.14	20.70
109 .....	2.20	1.80	11.41	5.30	4.90	4.50	3.10	15.50
103 .....	5.15	5.15	31.80	15.10	8.00	6.60	3.55	17.75
104 .....	5.15	.....	.....	.....	7.40	.....	.....	.....
107C .....	10.70	10.75	66.09	31.95	15.82	14.97	8.05	40.25
108C .....	10.80	.....	.....	.....	16.33	.....	.....	.....
W110 .....	8.60	8.82	52.98	25.50	16.10	15.00	8.06	40.30
W111 .....	9.05	.....	.....	.....	16.10	.....	.....	.....
Sucrose .....	23.50	.....	.....	.....	22.30	.....	.....	.....
	23.00	22.70	144.00	71.40	22.00	21.45	15.20	76.00
CARBON TREATED BEFORE INVERTASE HYDROLYSIS								
97 .....	17.10	16.55	105.00	51.50	18.80	17.85	12.31	61.55
98 .....	17.00	.....	.....	.....	17.70	.....	.....	.....
99 .....	18.30	17.70	112.20	55.20	20.40	19.80	13.65	68.25
100 .....	18.10	.....	.....	.....	20.00	.....	.....	.....
107 .....	6.10	5.60	35.50	16.90	7.00	6.60	4.50	22.50
109 .....	3.90	3.40	21.58	10.10	4.60	4.20	2.90	14.50
103 .....	5.90	4.95	30.50	14.40	8.34	6.47	3.48	17.40
104 .....	5.40	.....	.....	.....	6.81	.....	.....	.....
107C .....	11.00	10.50	64.61	31.30	15.20	14.80	7.96	39.80
108C .....	11.40	.....	.....	.....	16.60	.....	.....	.....
W110 .....	10.00	9.10	56.05	27.00	15.35	14.55	7.82	39.10
W111 .....	9.60	.....	.....	.....	15.95	.....	.....	.....
Sucrose .....	23.10	.....	.....	.....	22.00	.....	.....	.....
	23.70	22.80	144.50	71.60	21.70	21.15	15.00	75.00

obtained by the two methods. Although the data are meager, they are sufficient to show that for the samples tested the carbon had some effect on percentage of sugar whether run by the MUNSON-WALKER method or by the ceric sulphate method. Because of the varying results obtained and the large number of samples to be analyzed it was concluded that treatment with carbon would not be practical.

Since ceric sulphate was suitable for the analysis of reducing sugars and sucrose in plant tissue, it seemed reasonable to believe that it could be applied as a measure of polysaccharides which yield reducing substances upon

hydrolysis. Dry samples of the residue were therefore treated with taka diastase, cleared and delead, hydrolyzed with 1+20 hydrochloric acid, neutralized, and determinations made for reducing substances on aliquot portions with ceric sulphate. The acid hydrolyzable substances were also determined by this method. The results obtained for the polysaccharide fractions were very satisfactory and it is believed that materials which yield reducing substances upon hydrolysis may be determined by the use of ceric sulphate with results comparable to those obtained by copper reduction methods.

The values obtained by the two methods for total carbohydrates in white weed roots are given in tables I and II. The sum of sugars, starch (or diastase extract), and the acid hydrolyzable substances represents the total carbohydrates; these values are shown graphically in figure 4. Although

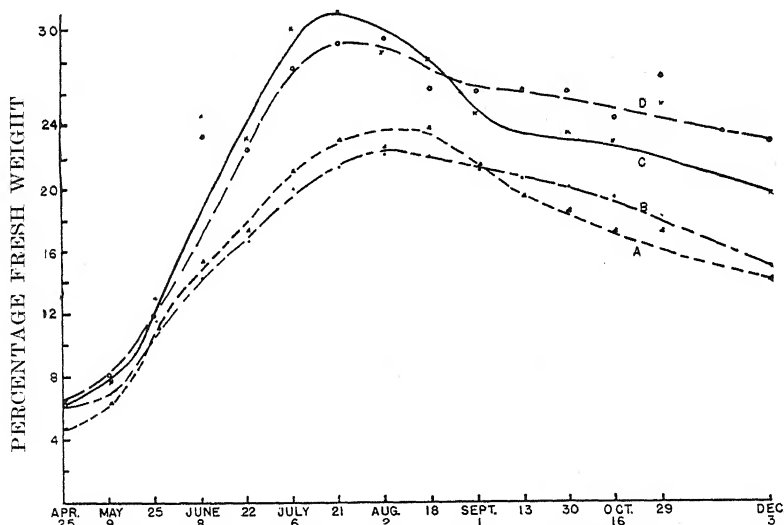


FIG. 4. Comparison of the ceric sulphate and MUNSON-WALKER methods for total carbohydrates in white weed roots. A—First foot level by the MUNSON-WALKER method. B—Second foot level by the MUNSON-WALKER method. C—First foot level by the ceric sulphate method. D—Second foot level by the ceric sulphate method.

values obtained by the ceric sulphate method were decidedly higher, especially when the carbohydrates were at the maximum for the season, the general trend of the carbohydrate content was the same by the two methods. For practical purposes in a weed-control program we believe that estimations of root reserves made with ceric sulphate give a sufficiently clear picture of the carbohydrate content.



### Summary

1. The method involving copper reduction was unsuitable for the determination of total sugars in the root extracts of *Lepidium* (white weed).

2. The method using ceric sulphate to determine carbohydrate content is discussed.

3. Data are presented which were obtained by the use of ceric sulphate in the determination of total sugars and total carbohydrates in the roots of *Lepidium*.

4. The data show close agreement between the ceric sulphate method and the MUNSON-WALKER method for reducing sugar content of bindweed roots. Reducing sugars and total sugars by ceric sulphate were from 1 to 5 per cent. higher than values obtained by the MUNSON-WALKER method.

5. Sucrose in root extracts of bindweed was determined with equal accuracy by either method.

6. Tests by both methods on samples treated with vegetable carbon in comparison with untreated samples gave varying results which made its use of little value in this investigation.

7. It is suggested that ceric sulphate may be used to obtain reliable values for carbohydrate fractions in root extracts of white weed and bindweed. The method has certain practical advantages: it is rapid, convenient, economical, and trustworthy.

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# EFFECT OF HEAVY AND LATE APPLICATIONS OF NITROGENOUS FERTILIZER ON THE COLD RESISTANCE OF KENTUCKY BLUEGRASS

J. C. CARROLL AND F. A. WELTON

## Introduction

The conditions under which turf-forming grasses on lawns and recreational fields are maintained are highly exacting. This is due chiefly to frequent and often close defoliation. On golf courses and athletic fields the situation is further aggravated by much trampling. To maintain a good sward under such adverse conditions resort is often made to liberal use of fertilizer. In order to take full advantage of the cool growing weather of late autumn and early spring, top-dressings are often made in the fall.

These applications are regarded as desirable where annual weeds such as crabgrass are a pest. Under such conditions spring and summer treatments may do more harm than good through undue stimulation of the weeds. In the judgment of many practical turf growers, however, late fall applications are detrimental because, in their opinion, they interfere with the normal "hardening off" of grass with the coming of cold weather and thus render it less resistant to the low temperatures of winter.

The purpose of the work reported in this paper was to determine whether or not the above assumption held by certain growers has any basis in fact. The investigation was comprised of two parts. (1) Certain physico-chemical determinations were made on unfertilized and heavily fertilized grass, determinations of constituents which are generally regarded as associated with winter hardiness in wheat. (2) The two classes of grass were exposed to varying degrees of artificial refrigeration and thus was determined, under comparable conditions, the resistance of each to low temperature.

## Materials and methods

For use in this study three lots of Kentucky bluegrass were produced: one for chemical analyses, and two for artificial refrigeration.

The grass for chemical analysis was obtained from a group of plots seeded August 15, 1928. On account of the small size of the plots (5 by 10 ft.) it was necessary to save clippings from several plots. Although each received its nitrogen through a different carrier, all were top-dressed to receive nitrogen at the same rate, 2.5 pounds per 1000 sq. ft. Applications at this rate were made three times in 1930: April 11, June 30, and September 15; and three times in 1931: April 21, July 29, and October 14.

In each season the plots were mowed at frequent intervals to maintain good lawn conditions. On account of extreme drouth, especially in 1930, infrequent mowing in midsummer sufficed. In addition to each fertilized plot there was an unfertilized plot of the same size which served as a check.

Supplemental material was obtained from a new seeding of Kentucky bluegrass made in August, 1936. A part of this area was fertilized with ammonium sulphate on September 10 and October 22. Both applications were made at the same rate as in the test already described. Samples from the unfertilized and fertilized areas were gathered from time to time during the late fall.

To test the relative resistance of unfertilized and fertilized Kentucky bluegrass to low temperatures a quantity of each kind was grown in gallon jars in a greenhouse in the winter of 1932-1933. The grass was grown in jars so that it could be transferred without disturbance of the roots to an artificial refrigeration room and there exposed to low temperatures. The jars were seeded at the rate of 5 pounds per 1000 sq. ft. Subirrigation was provided by means of a 0.75-inch glass tube placed at one side of the jar. The lower end rested on a layer of sand which covered the bottom of the jar. Some were fertilized with ammonium sulphate applied at the same rate as in the field tests. The first application was made when the grass was approximately 0.5 inch high; second and third applications were made at intervals of 6 and 12 weeks, respectively. The grass was clipped frequently to simulate lawn conditions and was allowed to develop in the greenhouse for approximately 6 months.

To test further the response of heavily fertilized Kentucky bluegrass to low temperatures samples were also taken from the new seeding made in the summer of 1936.

Samples for chemical analyses were collected from the older plots at irregular intervals during the seasons of 1930 and 1931 and from the new seeding during the late fall of 1936. They were taken between the hours of 10:00 and 11:00 A.M. after the grass was dry. In the fall and early winter it was necessary to use a broom in order to get rid of the heavy dew or frost with which the grass was coated. Immediately after clipping, the samples of grass were taken into the laboratory, ground finely with a food chopper, and thoroughly mixed. Suitable portions were taken for determinations of total moisture, free and bound water, expressible sap, total sugars, and pentosans.

Total moisture was determined on 50-gram duplicates by the toluene distillation method as modified by SAYRE and MORRIS (14). The free water was determined on three 25-gram samples by the calorimetric method first introduced by MÜLLER-THURGAU (7), and later improved by THOENES (17), ROBINSON (11), SAYRE (13), and MEYER (6). The tissue was frozen at

-25° C. for at least 3 days and the technique followed was essentially the same as that described by SAYRE (13). The bound water was obtained by difference.

Determinations of the sap expressible from fresh tissue were made on 100-gram duplicates. A hydraulic press and press cage of the type described by MEYER (6) and a pressure of 5000 pounds per square inch were used. A standard procedure was adopted which consisted of allowing the press cage to drain for 5 minutes and driving out with air the few drops remaining in the tube. Immediately after expression of the sap its specific gravity was determined with a hydrometer and its total solids by a refractometer. Duplicate portions (10 to 20 ml.) were preserved in alcohol for sugar analyses.

The total sugar content of the sap was determined from the alcohol-preserved samples by a modification of the QUISUMBING-THOMAS method (10), after the inversion of the sucrose by invertase, and calculated on a fresh weight basis. Pentosans were determined in the dried tissue by the method devised by YOUNGBURG (19) and modified by SUMINOKURA and NAKAHARA (16). Total nitrogen was determined in the dried tissue by the KJELDAHL-GUNNING method (5).

To determine the resistance of grass to cold, artificial refrigeration was employed. The limit of cold may, of course, be ascertained either by exposure of the grass for a definite period at progressively lower temperatures or by exposure at a definite temperature for varying periods of time. The former method was used on the grass grown in the greenhouse. Two jars each of unfertilized and fertilized grass were first placed in a hardening chamber at 0° C. for 10 hours. Although the time allowed for hardening was comparatively short, preliminary tests had shown it to be sufficient to cause an increase in bound water. The grass was then subjected to a definite sub-zero temperature for 8 hours. At the end of this period the minimum soil temperature was determined by means of a thermometer previously inserted in the soil to a depth of 1.5 inches. At the expiration of the period of exposure in the freezing chamber the grass was retransferred to the hardening chamber for 8 hours and thence to the greenhouse.

This procedure was repeated with similar groups of jars, each set being exposed to a lower temperature than the one preceding. Triplicate sets were exposed at each temperature. Observations were made on the stand of grass before, and again 3 weeks after, freezing. On the basis of these the percentage of survival was estimated.

To supplement the results obtained on the greenhouse grass, samples were taken from unfertilized and fertilized grass grown out-of-doors in the summer and fall of 1936. The samples were gathered in the fall on successive dates. The dates were not at regular intervals but were selected to

represent a progressive decline in temperature. The temperatures represented the general average that had prevailed during the preceding 5 days. In general the temperature of the individual days of each group was very uniform. By this procedure the grass was permitted to harden off naturally. In transferring the samples to the refrigerator, therefore, it was not necessary to first place them in a hardening chamber as was the case with those grown in the greenhouse; they were transferred directly to the refrigerator.

The procedure employed to expose the grass grown out-of-doors to artificial refrigeration was as follows. A sufficient number of vitrified gallon jars, approximately 6.5 inches in diameter and 7 inches deep, were first filled with Wooster silt loam, the same type of soil as that on which the grass was growing. The moisture content of this soil closely approximated that of the soil from the plots at the time the samples were taken. The soil was compacted firmly in the jars. This was important in order that the lowering of the temperature of the soil in the jars from the surface downward would proceed as uniformly as possible.

By means of a golf green cup hole digger, approximately 4.25 inches in diameter, a core of soil 4 to 5 inches in depth was removed from the center of each jar and replaced with a sample of sod of like size from the plots. The outer rim of soil in the jar was pressed firmly around the core of sod. The samples were then ready for exposure to low temperature in the refrigerator. On each date of freezing a definite number of cores (8 to 12) were lifted, packed in gallon jars, and placed directly in the refrigerator. A thermometer was inserted in the core to a depth of 1.5 inches to record the soil temperature. In this test the variability in exposure to cold was brought about by subjecting the grass to a constant temperature for different periods of time—the reverse of the process followed with the greenhouse grass. The refrigerator was regulated to maintain a constant air temperature of  $-25^{\circ}\text{C}$ . The removal of jars was based on a definite change in soil temperature rather than on exposure for a definite period of time. This was thought to be more accurate since previous experiments had shown that exposure of different jars for equal periods did not necessarily result in an equal lowering of the soil temperature. This was probably caused by variations in moisture content and compactness of the soil. As soon as a definite soil temperature had been reached two jars of both unfertilized and fertilized grass were removed from the refrigerator. Others of both classes were removed from time to time as the soil temperatures became progressively lower until finally all had been taken out. As fast as the jars were removed they were placed in a hardening chamber for 8 hours. At the expiration of that time they were taken to a greenhouse to allow time for recovery. Observations were made on the stand of grass before, and again

3 weeks after freezing. On the basis of these, the percentage of survival was estimated.

### Experimental results

In a study of this nature several bases on which the results may be interpreted present themselves. In the case of bound water, for example, early investigators chose to express it as percentage of the total moisture content. More recently other workers have expressed it in terms of weight of bound water per unit of dry matter. Again it might be expressed in terms of weight per unit of fresh tissue. With plant materials in which the content of moisture is considerably greater than that of dry matter, it is

TABLE I  
EFFECT OF NITROGENOUS FERTILIZER\* ON THE NITROGEN AND MOISTURE CONTENT  
OF KENTUCKY BLUEGRASS

DATE	TOTAL NITROGEN		TOTAL MOISTURE		FREE WATER		BOUND WATER	
	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N
	%	%	%	%	%	%	%	%
1930								
May 29 .....	0.86	1.21	65.4	73.3	37.8	50.9	27.6	22.4
June 12 .....	0.77	1.19	67.3	69.3	43.4	44.4	23.9	24.9
“ 26 .....	0.77	1.11	69.6	73.0	48.4	54.5	21.2	18.5
July 3 .....	0.81	1.40	60.4	68.2	44.7	51.8	15.7	16.4
“ 16 .....	1.12	1.83	58.0	58.2	41.4	41.1	16.6	17.1
Sept. 3 .....	0.99	1.11	76.8	78.0	57.9	64.2	18.9	13.8
“ 10 .....	0.73	1.31	68.7	72.4	52.2	58.2	16.5	14.2
“ 18 .....	1.10	1.33	67.6	73.6	53.6	55.9	14.0	17.7
“ 26 .....	0.97	1.11	69.6	73.6	52.2	67.2	17.4	11.4
Oct. 7 .....	0.92	1.33	66.4	73.2	49.0	60.8	17.4	12.4
“ 20 .....	0.92	1.30	66.8	74.8	58.6	61.1	18.2	13.7
Nov. 4 .....	0.86	1.24	64.0	72.2	44.0	55.0	20.0	17.2
Jan. 6, 1931 ..	.....	.....	63.0	69.4	44.8	54.4	18.2	15.0
1931								
May 15 .....	0.72	0.98	77.4	80.8	59.0	63.4	18.4	17.4
“ 29 .....	0.65	0.88	74.6	79.1	55.0	63.9	19.6	15.2
June 15 .....	0.84	1.00	72.3	77.4	59.4	66.4	12.9	11.0
July 2 .....	0.96	1.05	72.5	74.5	58.5	56.6	14.0	17.9
Sept. 16 .....	0.65	0.99	71.5	77.1	60.5	59.6	11.0	17.5
“ 25 .....	0.84	0.93	77.2	81.5	58.6	63.6	18.6	17.9
Oct. 13 .....	0.87	1.04	75.8	77.6	54.4	57.4	21.4	20.2
“ 21 .....	0.82	1.05	73.2	75.2	52.8	56.9	20.4	18.3
Nov. 3 .....	0.86	1.24	74.8	76.1	52.9	59.8	21.9	16.3
“ 28 .....	.....	.....	70.6	75.6	53.6	62.8	17.0	12.8
Jan. 7, 1932 ..	1.21	1.32	67.7	68.9	48.9	52.2	18.8	16.7
1936								
Oct. 20 .....	1.12	1.25	76.4	77.7	69.2	68.7	7.2	7.0
Nov. 12 .....	1.20	1.60	67.0	67.8	57.5	60.8	9.5	7.0
“ 16 .....	1.29	1.38	63.4	71.4	53.2	63.0	10.2	8.4
Dec. 1 .....	1.34	1.49	50.0	66.0	43.8	57.0	11.8	9.0

\* In this and in subsequent tables the results from the unfertilized and fertilized grass are recorded under the headings of “Low N,” and “High N,” respectively.

the opinion of the writers that the third method is to be preferred; it probably presents a clearer picture of the inner conditions of the living plant than either of the preceding. Accordingly, in this study the bound water and other constituents determined are expressed in terms of weight per unit of fresh tissue. The percentages of total nitrogen, total moisture, free water, and bound water found on the various dates of sampling in 1930, 1931, and 1936 are shown in table I.

From table I it is seen that the addition of nitrogenous fertilizer resulted in an increase in the content of total nitrogen on every date of sampling in each of the 3 years. Considered collectively for the 3 years, the increase amounted to approximately 30 per cent. The nitrogenous fertilizer resulted also in an increase in the content of total moisture on all of the dates

TABLE II

EFFECT OF NITROGENOUS FERTILIZER ON CERTAIN PHYSICOCHEMICAL CONSTITUENTS OF KENTUCKY BLUEGRASS

DATE	EXPRESSIBLE SAP		HYDRATION		TOTAL SUGARS		PENTOSANS (ARABINOSE)	
	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N	LOW N	HIGH N
	%	%	%	%	%	%	%	%
1930								
May 29 .....	26.7	29.0	149	178	2.07	1.92	3.34	1.58
June 12 .....	22.0	25.0	145	154	1.70	2.14	.....	.....
“ 26 .....	31.9	40.5	135	133	1.29	1.06	.....	.....
July 3 .....	31.0	32.9	84	122	2.48	1.81	2.77	2.82
“ 16 .....	16.1	19.2	105	99	2.43	2.52	.....	.....
Sept. 3 .....	37.3	38.3	181	193	1.57	1.07	.....	.....
“ 10 .....	23.2	25.3	125	170	.....	1.97	2.90	2.38
“ 18 .....	23.1	25.6	146	193	2.98	1.95	.....	.....
“ 26 .....	27.2	39.8	138	194	1.81	1.25	.....	.....
Oct. 7 .....	25.6	30.2	131	172	2.14	1.35	.....	.....
“ 20 .....	26.4	27.7	132	203	4.69	3.86	5.10	2.65
Nov. 4 .....	17.9	21.5	135	193	4.45	4.34	6.13	3.87
Jan. 6, 1931	4.5	12.9	160	191	4.82	4.48	.....	.....
1931								
May 15 .....	40.9	43.6	177	215	1.82	1.48	2.32	1.57
“ 29 .....	47.0	41.7	123	194	1.31	1.03	.....	.....
June 15 .....	42.4	40.9	119	175	1.36	1.36	.....	.....
July 2 .....	33.0	32.1	153	176	1.22	1.25	.....	.....
Sept. 16 .....	22.3	29.2	177	219	.....	.....	3.46	1.26
“ 25 .....	15.8	42.3	275	220	0.78	0.78	.....	.....
Oct. 13 .....	.....	.....	.....	.....	2.94	1.88	2.42	2.01
“ 21 .....	18.4	20.2	212	230	3.50	2.41	2.38	1.99
Nov. 3 .....	10.2	13.6	262	268	4.13	1.65	2.90	2.41
Jan. 7, 1932	6.2	10.6	193	193	4.56	2.09	3.70	3.11
1936								
Oct. 20 .....	25.5	30.0	227	272	2.25	2.00	1.92	1.82
Nov. 12 .....	9.8	12.2	179	181	3.58	3.61	2.64	2.02
“ 16 .....	14.5	18.9	140	193	5.34	3.57	3.42	2.00
Dec. 1 .....	0.0	7.5	133	177	5.50	3.92	6.62	3.09

of sampling. On the whole the increase amounted to approximately 7 per cent. Most of the increased moisture was in the form of free, or freezable water, since the free water was usually greater in the high- than in the low-nitrogen grass. On the other hand, the bound water was generally lower in the high- than in the low-nitrogen grass and no exceptions occurred in the fall after the coming of cool weather.

The percentages of expressible sap, hydration, total sugars, and pento-  
sans found on each day of sampling in the 3 years, 1930, 1931, and 1936, were as shown in table II.

From table II it is seen that the percentage of expressible sap was almost always higher in the high- than in the low-nitrogen grass. The difference was usually greater in the cool weather of fall than in midsummer. In one year, 1931, it was actually less for a time in the summer. Likewise, the percentage of hydration was, with few exceptions, higher in the high- than in the low-nitrogen grass. On the other hand, the total sugars and pento-  
sans were generally higher in the low- than in the high-nitrogen grass.

The response of the low- and high-nitrogen grass to low temperatures is shown by the refrigeration tests. As previously stated, variability in exposure of grass grown in the greenhouse was effected by changing not the time of exposure but the temperature. The air temperatures to which the grass was exposed, the lowered temperature of the soil, and the percentage of survival are shown in table III.

TABLE III  
RESULTS OF FREEZING KENTUCKY BLUEGRASS GROWN IN A GREENHOUSE

AIR TEMPERATURE	FINAL SOIL TEMPERATURE	SURVIVAL AFTER 2 WEEKS	
		LOW N	HIGH N
° C.	° C.	%	%
-12	-6	95	50
-14	-11	80	45
-16	-14	10	5
-18	-16	10	0

It is evident that the high-nitrogen was more susceptible than low-nitrogen grass to low temperatures since at each sub-zero temperature the percentage of survival was lower. Moreover, the lower the temperature to which the air and soil descended the less was the survival of both classes of grass.

To supplement the results obtained on the greenhouse grass, samples were collected from unfertilized and fertilized grass grown out-of-doors. The date of sampling (accompanied by the hardening temperatures), the scale of descending soil temperatures used, and the percentage of survival



TABLE IV  
RESULTS OF FREEZING KENTUCKY BLUEGRASS GROWN OUT-OF-DOORS

FINAL SOIL TEMPERATURE AFTER EXPOSURE FOR VARIOUS PERIODS IN REFRIGERATOR	DATE OF SAMPLING—1936														
	OCTOBER 20 (+ 6.2° C.) *				NOVEMBER 12 (- 2.2° C.) *				NOVEMBER 16 (- 2.6° C.) *				DECEMBER 1 (- 9.6° C.) *		
	EXPO- SURE AT - 25° C.	SURVIVAL			EXPO- SURE AT - 25° C.	SURVIVAL			EXPO- SURE AT - 25° C.	SURVIVAL			EXPO- SURE AT - 25° C.	SURVIVAL	
		Low N	High N	%		Low N	High N	%		Low N	High N	%		Low N	High N
°C.	hr.	%	%	hr.	%	%	hr.	%	%	hr.	%	%	hr.	%	%
0°	2.5	100	100	2.25	100	100	2.0	100	100	2.75	100	75	3.0	100	45
- 1°	4.0	50	50	4.5	100	75	3.0	100	50	3.0	100	25	3.5	95	10
- 3°	5.0	0	0	.....	75	5	3.5	85	5	4.0	80	5	4.25	60	5
- 5°	5.25	0	0	5.0	25	0	4.0	.....	.....	5.0	40	.....	.....	.....	.....
- 7°	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- 9°	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- 11°	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
- 13°	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....

\* The figures in parentheses accompanying each date of sampling represent the average minimum air temperature for 5 days previous to sampling and, hence, the hardening temperatures to which the grass had been exposed at the time of cutting.

of the low- and high-nitrogen grass are shown in table IV. These results indicate no difference in the resistance of low- and high-nitrogen grass on the first date of sampling. The absence of such difference was probably due to the lack of hardening, since investigators (1, 2, 18) have shown that most plants do not begin to harden until the temperature descends to approximately  $+6^{\circ}\text{C}$ . Prior to October 20, 1936, the minimum temperature had seldom descended to that point. Moreover, the physico-chemical composition of the grass shown in tables I and II for 1936 failed to show important differences at that time. From November 4, 1936, until after the last sampling date of December 1, the temperatures were all well below the threshold for hardening, usually below  $0^{\circ}\text{C}$ . The maximum temperatures for a majority of the days were likewise within the hardening range. Further examination of table IV shows that the resistance to cold of both the low- and high-nitrogen grass becomes progressively greater as the hardening temperatures lower. The resistance of the low-nitrogen grass, however, was markedly higher than was that of the high-nitrogen grass. It is pertinent to note that the lower the temperature at the time of sampling the lower the temperature of the soil before killing occurred.

### Discussion

Artificial refrigeration of Kentucky bluegrass grown in a greenhouse and hardened in a low temperature chamber showed that high- was more susceptible than low-nitrogen grass to sub-zero temperatures. Likewise, artificial refrigeration of Kentucky bluegrass grown out-of-doors and hardened naturally by gradually lowering temperatures during the fall months, showed that high- was more susceptible than low-nitrogen grass to sub-zero temperatures. In both cases the relatively high nitrogen content of the high-nitrogen grass was induced by heavy and repeated applications of ammonium sulphate (2.5 pounds N per 1000 sq. ft.). The top-dressings on grass grown out-of-doors were made in September and October. The refrigeration tests, therefore, indicate that heavy and late applications of nitrogenous fertilizer to Kentucky bluegrass reduce its resistance to sub-zero temperatures.

The temperatures at which the grass succumbed were higher than those to which it is exposed in most winters in this latitude. In nature, however, it is probable that grass would offer more resistance than in the highly artificial conditions under which it was exposed in these tests. Inasmuch as both classes of grass were exposed under the same conditions the results are comparable and, therefore, represent the relative resistance of the two classes of grass.

Some indication of the reason for the relatively low resistance of the high- as contrasted with the low-nitrogen grass is afforded by the physico-

chemical results shown in tables I and II. Unfortunately, only the grass sampled in 1936 was grown on the same plots and under the same conditions as that which was exposed to low temperatures. Inasmuch as the greenhouse grass was grown on soil of the same type and of equal fertility, and was fertilized with the same frequency and with the same quantity of nitrogen as was that grown out-of-doors, it is probable that the internal responses were similar; therefore, its physico-chemical composition was practically the same as that grown out-of-doors. Assuming this to be the case, the results indicate that applications of nitrogenous fertilizer in the fall, when the grass is naturally "hardening off," reduce the elaboration of hydrophilic colloids. The expressible sap during that period is consistently higher in the high-nitrogen grass than in the low-nitrogen grass. On the contrary, the bound water is consistently lower. These findings are in agreement with those of NEWTON (8, 9) and MARTIN (4), who reported similar differences between non-hardy and hardy varieties of wheat.

The percentage of hydration, that is, the residual water content after expressing the sap (expressed as a percentage of the dry weight), seemingly presents a theoretical contradiction to the conclusions drawn from the relative weights of expressed sap. NEWTON (8), however, concluded that it was a less dependable measure of hardness than relative amounts of sap expressed.

Among the hydrophilic colloids which have been considered by a number of workers (3, 12, 15) to play a prominent part in hardness are the pentosans. The determinations made in this study show that the applications of nitrogen decrease the formation of pentosans in Kentucky bluegrass. The results of these determinations are reported as pentose, since a study of the optical properties<sup>1</sup> of the phenylosazone derivative of the products of acid hydrolysis of the dried tissue proved it to be arabinose. Analyses made on scattered samples showed that only 1 to 2 per cent. of the pentose present was contained in the expressed sap. This is to be expected since such material is found chiefly in the cell wall.

The results also show that with the advent of colder weather there is an increase in the total sugar content of both the low- and high-nitrogen grass. In the low-nitrogen grass, however, the increase is significantly greater. The exact rôle that an increased sugar content plays in the cold resistance of plants is not definitely known. The osmotic pressure of the cell sap is increased and this added pressure increases the resistance of the cell to the withdrawal of water during freezing. The fact, however, that the sugars exert a protective action against frost precipitation of the proteins is probably more significant.

<sup>1</sup> The authors are indebted to Dr. V. H. MORRIS of the Ohio Agricultural Experiment Station for this identification.

### Summary

1. Heavy and late applications of nitrogenous fertilizer to Kentucky bluegrass increased the content of total nitrogen and total moisture on every date of sampling, which included two full seasons and a part of a third.

2. The same treatment resulted generally in a higher content of free water, expressible sap, and a higher percentage of hydration in the high- than in the low-nitrogen grass. Usually the differences were less in the summer than in the fall after the coming of cold weather.

3. The same treatment resulted generally in a lower content of bound water, total sugars, and pentosans in the high- than in the low-nitrogen grass. Usually the differences were much accentuated with the coming of cool weather in the fall.

4. Artificial refrigeration of both classes of grass in the unhardened condition showed no difference in resistance to cold.

5. Artificial refrigeration of both classes of grass after hardening showed that the high-nitrogen grass was less resistant than the low-nitrogen grass to low temperatures.

6. Inasmuch as the difference in nitrogen content of the Kentucky bluegrass was induced by heavy and late applications of nitrogenous fertilizer, it follows that such a practice may be expected to lessen somewhat the resistance of Kentucky bluegrass to low temperatures.

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# SUGGESTIONS FOR THE USE OF WARBURG RESPIROMETERS IN PLANT PHYSIOLOGICAL INVESTIGATIONS<sup>1</sup>

JAMES W. BROWN

(WITH TWO FIGURES)

## Introduction

Although Warburg respirometers have been used extensively in animal physiology for a number of years they have not been employed by plant physiologists as much as their accuracy and convenience seem to warrant. HARRINGTON (2) used a modification of the Warburg principle in his respirometers, but TANG (6) and MURLIN (5) apparently are the only workers to use the standard type of respirometer for studies of seed respiration. The Warburg respirometer permits simultaneous measurement of  $O_2$  consumption and  $CO_2$  production on small samples for short time intervals. This makes possible easy and rapid replication of determinations without excessive use of material. By the use of respirometer vessels of suitable size and shape not only seeds, but small fruits, small seedlings, excised roots, buds, flowers and other plant parts may be placed in them.

A careful study of the technique was made in connection with their use in an investigation of the respiration of acorns at various temperatures. It was found that several factors must be considered which are not included in the technique as given in the papers describing their operation (4, 5, 6, 7). This paper suggests certain modifications of the standard procedure together with suggestions on the operation of both large and small respirometer vessels.

## Experimental methods

The usual procedure for obtaining concurrent measurements of  $O_2$  consumption and  $CO_2$  production is as follows. The time periods given are those which the writer found necessary for the conditions of his investigation and the letters refer to the sample record sheet at the end of the paper. For the most efficient operation of the respirometers it is desirable to determine experimentally the time periods required for temperature adjustment. This can be done by placing the vessels in the bath under the conditions of the experiment and observing the time necessary to bring the manometric fluid to a constant level after the stopcocks of the manometers are closed.

An acid, usually HCl, is placed in the side arm of the respirometer vessel which is then stoppered. An alkali, usually NaOH or KOH, is placed in the bottom of the vessel or in the central cup if one is present, the support

<sup>1</sup> No doubt some investigators have used most of the suggestions offered in this paper, but since they have never been published in any collected form, they are presented here mainly for the consideration of those who are just beginning to use the method.

for the respiring material is placed in position, the top of the vessel seated and the whole unit attached to the manometer. The apparatus is set upon a shaker so that the vessels are submerged in a constant temperature bath. After the vessel has come to the temperature of the bath (7 minutes) the apparatus is removed from the shaker, taken apart, the respiring material placed in the vessel upon the support, and the unit reassembled and returned to the shaker. The time  $a$  is recorded when the material is placed in the vessel, and this time is used as the start of the period of  $\text{CO}_2$  production. Three minutes later, after respirometer and material are at the temperature of the bath, the manometric fluid is levelled to the 150-mm. mark in the arm connected to the vessel and the level in the open arm is recorded,  $b$ , with the time  $c$ , and the stopcock is closed. The time  $c$  marks the start of the period of  $\text{O}_2$  consumption. The apparatus is left on the shaker for the length of the experiment (30 minutes). The shaker was operated at 70 complete oscillations per minute with a throw of 7 cm. Whenever a reading is made care must be taken to have the manometers in a vertical position.

When the period of  $\text{O}_2$  consumption is ended, the time  $d$  is recorded and the manometric fluid is again brought to the 150-mm. mark and the open arm reading  $e$  is made. The length of the  $\text{O}_2$  consumption period is then  $d - c$ , while the change in manometric graduations,  $b - e$ , represents the amount of  $\text{O}_2$  consumption. The apparatus is taken from the shaker immediately after recording  $d$  and  $e$  and turned in such a position as to cause the acid from the side arm to flow into the alkali in the vessel and liberate the  $\text{CO}_2$  which has been absorbed by the alkali. An excess of acid is necessary and where very small amounts of the reagents are used it is advisable to tilt the unit so that the alkali flows into the side arm and then allow the mixture to return to the vessel to insure more thorough mixing of all of the reagents. The apparatus is then replaced on the shaker and after a period of three minutes the level of the fluid in the closed arm of the manometer is brought again to the 150-mm. mark and a reading of the level in the open arm,  $f$ , is recorded with the time  $g$ . The time  $g$  marks the end of the period of  $\text{CO}_2$  production,  $g - a$ , and the "apparent" amount (see corrections 1 and 3) of  $\text{CO}_2$  produced is represented as the change in level of  $f - e$  of experimental respirometer minus  $f - e$  of the thermobarometer which is substituted for  $h$  in the following formula. The volume of the respiring material is determined and allowance for this volume is made in the formula. At the end of the experiment the dry weight of the material is determined if practicable in order to place the results on a dry weight basis.

Whenever a series of determinations is to be made a control respirometer (thermobarometer, check, or blank) must be subjected to the same conditions, reagents, and treatment as the series except that it contains no respir-

ing material. This provides a correction for any temperature and external pressure changes during the run, and for any  $\text{CO}_2$  which may be dissolved in the reagents before the experiment.

The following formula is used to calculate the volumes of gas absorbed and produced by the respiring material.

$$x = h \left[ \frac{V_g \frac{273}{T} + V_f (\alpha)}{P_o} \right]$$

where:

$x$  =  $\text{mm.}^3$  of gas at S. T. P.

$h$  = height change in manometric graduations,  $b - e$ .

$V_g$  = free volume of gas in the vessel and manometer to the level of manometric fluid (total volume of apparatus less the volume of the sample, liquids and detachable supports placed in the vessel). See DIXON (1).

$T$  = absolute temperature of the water bath surrounding the vessel.

$V_f$  = the volume of all liquids in which the measured gas might dissolve.

$\alpha$  = the solubility of the gas being measured in the contained liquids at the temperature  $T$ . BUNSEN's solubility table has been recommended (4),  $V_f (\alpha)$  gives the volume of the dissolved gas and this is added to the free gas to give the total volume (this expression was omitted by the writer for reasons presented later in this paper).

$P_o$  = normal pressure in terms of manometric fluid (for BRODIE's solution, 10,000 mm.).

In experiments where a constant volume of material is to be used at constant temperature, the quantity within the brackets in the above formula remains constant for a given vessel and can be assigned a value which may be referred to as the vessel constant. The vessels used in the present work

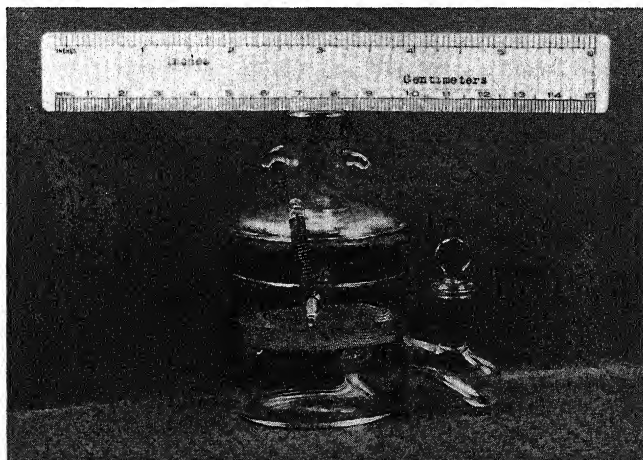


FIG. 1. Modified Warburg respirometer having a volume of approximately 90 ml.



had a capacity of about 90 ml. (fig. 1) and vessel constants of from 7 to 9 whereas the majority of vessels described in the literature are of about 15-ml. volume and have correspondingly smaller vessel constants of about 1 to 3. Where the vessel constant of an apparatus is 1 the reading of a change in pressure on the manometer may be transferred directly to terms of volume; but as the vessel constant increases, the change in pressure as shown by the manometer is smaller by the reciprocal of the vessel constant. When a vessel constant becomes as large as 10, the accuracy with which the reading may be interpreted becomes 0.1 as great as if the vessel constant were 1, or each manometric graduation would represent an internal change of 10 mm.<sup>3</sup>, whereas with a vessel constant of 1 it represents an internal change of 1 mm.<sup>3</sup>

The use of the large vessels has made evident certain factors that apparently have frequently been overlooked in calculating the results of determinations made with the smaller vessels. Some of these factors are equally important, however, for small and large vessels.

#### CORRECTION FOR INTERFERENCE OF O<sub>2</sub> CONSUMPTION WITH CO<sub>2</sub> PRODUCTION READINGS

In previous investigations involving the simultaneous determination of O<sub>2</sub> absorption and CO<sub>2</sub> production with the Warburg apparatus the fact apparently has been overlooked that these two processes are continuously concurrent. The usual technique (4, 5, 6) does not measure both processes for the same period of time.

During most of a determination all CO<sub>2</sub> produced is absorbed by alkali in the bottom of the vessels so any change of volume is caused by consumption of O<sub>2</sub> by the respiring material. At the end of this period the manometer is read and the acid in the side arm mixed with the alkali to release the absorbed CO<sub>2</sub>. After several minutes the manometer is read again and all change in pressure is attributed to the CO<sub>2</sub> produced by respiration and that already in the reagents. Oxygen consumption, however, has continued during this period so in reality the change in pressure represents that produced by CO<sub>2</sub> minus the O<sub>2</sub> absorbed during the period required for mixing the reagents and again bringing the temperature of the vessels into equilibrium with that of the bath. It is therefore quite evident that in order to obtain the actual volume of CO<sub>2</sub> produced the volume of O<sub>2</sub> absorbed during the period of mixing and temperature adjustment must be added to the apparent volume of CO<sub>2</sub> and the thermobarometer correction applied. This is particularly important where a low R. Q. (respiratory quotient =  $\frac{\text{CO}_2}{\text{O}_2}$ )

exists and also where the time interval is relatively long. Under ordinary conditions the time interval required for mixing reagents and returning the

vessels to the temperature of the bath is 3 to 5 minutes which is 10 to 15 per cent. of the time for which  $O_2$  consumption is being measured. The situation is represented diagrammatically in figure 2. The manometer read-

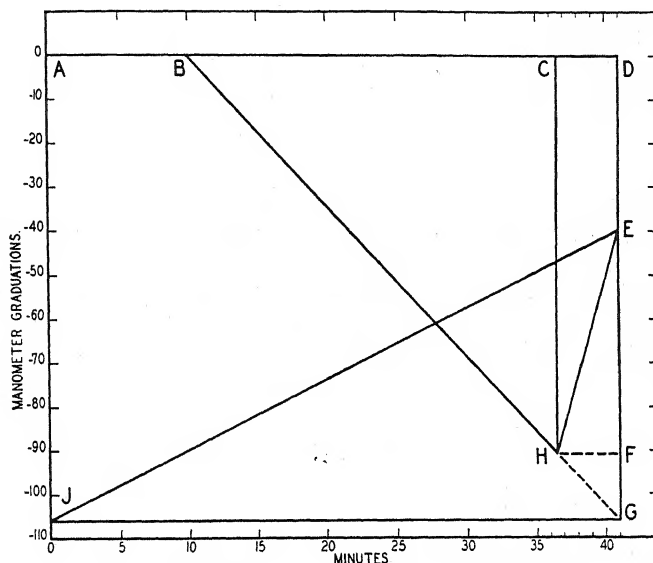


Fig. 2. Suggested procedure compared with usual procedure for obtaining a true reading of  $CO_2$  production.

BH—Progress of  $O_2$  consumption during experiment. Measured at H in 26.5 minutes (BC) by line CH.

H—Point at which reagents were mixed to liberate  $CO_2$ . Used by most workers as base for obtaining  $CO_2$  readings.

E—Point at which  $CO_2$  production is measured by difference (vertical or EF) between basic reading at H and final reading at E.

EF— $CO_2$  production for 41 minutes measured by generally accepted method.

HG—Extrapolation of BH showing estimated progress of  $O_2$  consumption while waiting for reading of  $CO_2$  production at E.

G—Point  $O_2$  consumption is estimated to have reached at time of reading E.

FG—Additional  $O_2$  consumption while waiting to make reading E. Should be added to EF to make correct  $CO_2$  reading.

G—Base which should be used instead of H for correct  $CO_2$  readings.

JG—Length of time  $CO_2$  production is measured. Same as AD. Should be used as base line (zero point) for measuring  $CO_2$  produced during the length of the experiment.

JE—Progress of  $CO_2$  production. When used in conjunction with JG it is possible to make readings of  $CO_2$  at any point during experiment.

BG—Progress of  $O_2$  consumption which makes possible a determination of  $O_2$  consumed at any time during the experiment.

ing for  $O_2$  consumed (fig. 2) in 26.5 minutes is 91 (CH) or approximately 141 for 41 minutes. By the usual method the manometer reading for  $CO_2$  production is 51 (EF) for 41 minutes but by the suggested procedure becomes 66.3 for the same period. Disregarding the solubility of these gases for reasons presented later, the R. Q.'s differ as follows:

$$\frac{51}{141} = 0.36 \text{ R. Q. by the usual procedure}$$

$$\frac{66.3}{141} = 0.47 \text{ R. Q. by the suggested procedure}$$

#### CORRECTION FOR SOLUBILITY OF GASES

A fault of the usual technique exists in the method of correcting for the solubility of gases in the reagents. Theoretically, the solubility of  $O_2$  and  $CO_2$  in the reagents should be considered, but in most experiments it is a negligible factor and correction for solubility by the usual method may actually decrease the accuracy of the measurements. The usual procedure suggests the use of Bunsen's solubility tables (3) but these tables represent determinations using distilled water as the solvent and the gas at 760 mm. of pressure. Neither of these conditions holds for the respiration experiments, for the final solution in the respirometer is a salt solution in which gases are less soluble than in pure water and the concentration of gases, especially  $CO_2$  is low. In the writer's experiments the concentration of  $CO_2$  in the vessels did not exceed three times its concentration in the normal atmosphere and its pressure would be only about 0.76 mm. or 0.1 per cent. of that called for by standard solubility tables. The alkali used by the writer for absorption of  $CO_2$  was 1.5 ml. of 2N NaOH and the acid used to liberate the  $CO_2$  was 0.6 ml. of 6N HCl. Actually there was much more  $CO_2$  in the alkali before it was placed in the vessels than in the salt solution at the end of a determination, hence none of that produced in respiration could be lost by absorption in the reagents.

Both reagents have been exposed to the  $O_2$  of the air hence are in equilibrium with the concentration of  $O_2$  existing in the vessels at the beginning of the experiment. Since only a very slight change in  $O_2$  pressure occurs during an ordinary determination, solubility of  $O_2$  may also be safely disregarded.

If the vessel constants are corrected for solubility by using the standard solubility tables they are increased. Thus, the product of the vessel constant and the manometric reading is also increased. It happens that the extent of the increase of  $CO_2$  production corrected on the basis of the solubility tables in the usual procedure approaches the increase obtained by the suggested procedure (fig. 2) and the final volume of  $CO_2$  found by both methods may be nearly equal in some cases. The  $O_2$  reading, however, is invariably higher when the solubility tables are used in the usual procedure. The attempt to obtain more accurate results by correcting for solubility of gases by these tables, therefore, actually decreases their accuracy. For these reasons the writer did not use the expression  $V_r (\alpha)$  in the formula presented earlier.

CORRECTION FOR DIFFERENCE BETWEEN VOLUMES OF THERMOBAROMETER  
AND OTHER VESSELS

In making corrections for pressure changes in the vessels as indicated by the behavior of the thermobarometer certain additional corrections are necessary.

During the period in which  $O_2$  absorption is measured there is no change in the mass and volume of gas contained in the thermobarometer even though it may be subjected to temperature and pressure changes. This situation holds for any empty respirometer. The manometric change caused by external conditions for a period of time will be equal in all respirometers regardless of size and content. This equality is evident when the respirometers are run empty but it still exists when they contain respiring material. The thermobarometer correction for pressure and temperature changes during the period of  $O_2$  consumption can therefore be applied directly to the determination of changes in  $O_2$  volume of all respirometers. As the temperature decreases, or the external pressure increases, the manometric reading of the respirometers will decrease; and as the temperature increases or the external pressure decreases the manometric reading of the respirometers will increase. Thus, the thermobarometer correction may be applied by either subtracting the decrease of the thermobarometer reading from, or adding the increase of the thermobarometer reading to, the reading of each of the experimental respirometers.

The correction for  $CO_2$  contained in the reagents cannot be applied directly to the other respirometers, however, unless they all contain equal volumes of gas, because the introduction of equal volumes of gas into systems of different volumes will produce different changes in pressure. If, for example, the total volume of one system is 500 ml. and the volume of another system is 100 ml., an addition of 10 ml. of gas to each of the systems will cause the pressures of those systems to change. The extent of these changes will be  $\frac{10}{500}$  and  $\frac{10}{100}$ .

$$\frac{10}{500} \times 760 = 15.2 \text{ mm. increase in pressure or } 775.2 \text{ mm. total pressure.}$$

$$\frac{10}{100} \times 760 = 76.00 \text{ mm. increase in pressure or } 836.00 \text{ mm. total pressure.}$$

Thus it is clear that the pressure change in the 500-ml. system is less than the pressure change in the 100-ml. system when equal volumes of gas are introduced into them.

Table I shows the relation between changes of manometric readings of the thermobarometer and the corresponding changes of each of the individual respirometers. This table is based on the values obtained by using

TABLE I

MANOMETRIC READINGS FOR EQUAL QUANTITIES OF GAS INTRODUCED INTO RESPIROMETER SYSTEMS OF DIFFERENT VOLUMES

	CONTROL RESPIROM- ETER (THERMO- BAROMETER)	RESPIROM- ETER 1	RESPIROM- ETER 2	RESPIROM- ETER 3	RESPIROM- ETER 4
Volume in mm. <sup>3</sup> .....	90512	94686	90475	88945	99885
Change in mano- metric read- ing in mm. {	30	28.68	30.00	30.53	27.18
	60	57.36	60.00	61.06	54.36
	90	86.04	90.00	91.59	81.54

the volume of the control respirometer as the numerator and the volume of the respirometer in question as the denominator.

As has been shown, the larger volume would be affected least by an addition of a unit of gas, thus:  $\frac{90512}{99885} = 0.906$ , showing that a change of 1 mm. of the control manometer would be analogous to a change of 0.906 of the manometer of respirometer 4. Of course, the larger the volume of gas which is introduced into the systems becomes, the more conspicuous this difference becomes, until a change of 90 mm. of the control manometer would indicate a change of only 81.54 mm. in the manometer of respirometer 4.

As the volumes of the vessels become smaller the differences between the volumes of the control and the experimental respirometers must become proportionately smaller if the ratio between the volumes is to be kept near 1. Should the control respirometer and an experimental respirometer have respective total volumes of 9 and 10 ml., the correction factor for the experimental respirometer would be  $\frac{9}{10} = 0.9$ , or practically the same as the correction factor obtained in the preceding paragraph where the difference in volume of the two systems is 10 ml. while in the present instance the difference is but 1 ml.

It is important to note that a complete table of the type suggested by table I cannot be constructed unless a constant volume of material is to be used. In such an instance the volume of the material and reagents should be deducted from the total volume of the systems of the experimental respirometers. The volume of the reagents contained in the thermobarometer should also be deducted from its total volume.

Suppose, during the experiment, the control respirometer (10-ml. volume) showed a manometric reading of 30 for CO<sub>2</sub> and 1 ml. of the reagents and respirometer no. 1 (12-ml. volume) showed a manometric reading of

80 for the  $\text{CO}_2$  produced by 1 ml. of respiring material plus the amount contained in 1 ml. of the reagents. The usual procedure has been to subtract 30 from 80 for a reading of the  $\text{CO}_2$  produced by the respiring material. The fallacy in this is shown by the following calculations.

The vessel constants of the systems are computed for  $0^\circ \text{C}$ . using the formula which has been given.

$$\frac{(10 - 1) \times \frac{273}{273}}{10} = 0.9 \text{ for the control respirometer and}$$

$$\frac{[12 - (1 + 1)] \times \frac{273}{273}}{10} = 1 \text{ for respirometer no. 1.}$$

$80 - 30 = 50$  manometric graduations of respirometer no. 1.

$50 \times 1$  (vessel constant of no. 1) =  $50.0 \text{ mm.}^3 \text{ CO}_2$  by the usual method.

A change of 30 graduations of the control, however, would be equal to  $0.9 \times 30$  graduations of no. 1, therefore:

$80 - (0.9 \times 30) = 53$  manometric graduations of no. 1

$53 \times 1 = 53.0 \text{ mm.}^3 \text{ CO}_2$  by suggested procedure

or if one prefers to deal with actual volumes of gas instead of manometric graduations the conversion for the same instance will be:

$(80 \times 1) - (30 \times 0.9) = 53.0 \text{ mm.}^3 \text{ of CO}_2$ .

The difference shows

$$\frac{(53 - 50)}{53} \times 100 = 5.66 \text{ per cent. too little CO}_2 \text{ measured by the usual procedure.}$$

In an experiment using a control respirometer of 92 ml. and an experimental respirometer of 112 ml., suppose the control showed a manometric reading of 30 while the manometer of experimental respirometer no. 2, which contained 10 ml. of respiring material plus 2 ml. of reagents, read 80 for the production of  $\text{CO}_2$  at  $0^\circ \text{C}$ .

Then:

$$\frac{(92 - 2) \times \frac{273}{273}}{10} = 9, \text{ the vessel constant for the control,}$$

and

$$\frac{[112 - (10 + 2)] \times \frac{273}{273}}{10} = 10, \text{ the vessel constant for no. 2.}$$

The conversion factor in this instance is the same as in the previous experiment for  $\frac{90}{100} = 0.9$ , therefore:

$0.9 \times 30 = 27$

$80 - 27 = 53$  manometric graduations of no. 2

$53 \times 10 = 530 \text{ mm.}^3 \text{ of CO}_2$  by the suggested procedure

and

80 - 30 = 50 manometric graduations of no. 2  
 50 × 10 = 500 mm.<sup>3</sup> of CO<sub>2</sub> by the usual procedure.

The difference shows

$\frac{530 - 500}{530} \times 100 = 5.66$  per cent. too little CO<sub>2</sub> measured by the usual procedure.

In this second instance the percentage difference is the same but the amount of gas concerned is ten times as much as in the first instance. It may be repeated that the smaller the vessel constant of a system, the greater is the accuracy of manometric readings for that system. While the effect of the difference in volume between the control and experimental respirometers may be considered negligible in some studies (when the vessels are of 10 to 15 ml. capacity), in investigations where larger (100-ml.) respirometers are used the effect of this difference in volume would necessarily be considered in order to obtain the greatest possible accuracy for the experiment.

### Summary

A study of the operation of Warburg respirometers has been made in connection with an investigation of the oxygen consumption and carbon

#### SAMPLE RECORD SHEET

	EXPERIMENTAL RESPIROMETER		THERMOBAROMETER OR CONTROL	
Dry wt. of sample in grams .....	10.4476			
Volume of sample and /or reagents in ml. ....	17.6		2.1	
Volume of system in experiment in mm. <sup>3</sup>	87810 - 17600 = 70210		90512 - 2100 = 88412	
	TIME	READING	TIME	READING
Set in bath .....	9: 55		9: 55	
Seeds placed in vessel, start CO <sub>2</sub> .....	10: 02 (a)			
Stopcock closed, start O <sub>2</sub> .....	10: 05 (c)	150.5 (b)	10: 05 (c)	149 (b)
End O <sub>2</sub> , dump HCl .....	10: 35 (d)	123 (e)	10: 35 (d)	151 (e)
End CO <sub>2</sub> , end of mixing	10: 38 (g)	167 (f)	10: 38 (g)	181.5 (f)
	EXPERIMENTAL RESPIROMETER GRADUATIONS	CONTROL CORRECTION FOR EXPERIMENTAL RESPIROMETER	CONTROL	
Change, O <sub>2</sub> .....	150.5 - 123 = 27.5	2	151 - 149 = 2	
Corrected O <sub>2</sub> .....	27.5 + 2 = 29.5			
Change, CO <sub>2</sub> .....	167 - 123 = 44	$\frac{88412}{70210} \times 30.5 = 38.40$	181.5 - 151 = 30.5	
Plus O <sub>2</sub> consumed in 3 minutes .....	(44 - 38.40) + 2.95			
Corrected CO <sub>2</sub> .....	8.55			

## CALCULATIONS

VESSEL CONSTANT (0° C.)	× MANOMETER GRADUATIONS	× TIME CORRECTION	= TOTAL GAS IN MM. <sup>3</sup> (30 MIN.)
$\frac{87810 - 17600}{10000} = 7.021$	8.55	$\frac{30}{36} = 0.8333$	50.02 mm. <sup>3</sup> CO <sub>2</sub>
7.021	29.5		207.12 mm. <sup>3</sup> O <sub>2</sub>

$$\frac{\text{Total CO}_2}{\text{Total O}_2} = R. Q. \text{ or } \frac{50.02}{207.12} = 0.2415$$

TOTAL GAS	÷ DRY WT. OF SAMPLE IN GM.	× TIME CORRECTION	= MM. <sup>3</sup> OF GAS PER GM. DRY WT. PER HOUR
50.02	10.4476	2	= 9.58 mm. <sup>3</sup> CO <sub>2</sub>
207.12	10.4476	2	= 39.64 mm. <sup>3</sup> O <sub>2</sub>

dioxide production of acorns. It was found that the accuracy of the method could be increased by modifying the usual technique in three ways. A correction should be made for the absorption of oxygen during the interval between the final oxygen reading and the final carbon dioxide reading. Theoretically, a correction should be considered for the solubility of these gases in the reagents before and after mixing, but since reliable solubility values are frequently not available for these reagents more accurate results may be obtained by omitting a correction for solubility. Differences in volume of respirometers prevent the direct application of a control correction for carbon dioxide in the reagents. A sample set of recordings and calculations are presented.

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## RELATION BETWEEN FRUIT SIZE AND FOOD SUPPLY IN THE TOMATO<sup>1</sup>

FELIX G. GUSTAFSON AND HELEN B. HOUGHTALING  
(WITH FIVE FIGURES)

Recently GUSTAFSON and STOLDT (2) made a study of the relation between leaf area and fruit size of the tomato. In this study it was found that by increasing the leaf area per fruit, the size of the fruit produced was also increased. In their experiments the leaf area was not adjusted until after the ovary had completed its growth and the fruits had set. It has therefore seemed desirable to attempt to influence the size of the fruit while the ovary is being formed as well as during the period of its development into a fruit. In the present study we have measured the volume of the ovaries at the time of blossoming, the volume of the mature fruits, the cell size of these ovaries and mature fruits, and the diameter of the pith of the stem just below the insertion of the flower cluster; and we have also counted the number of locules in the fruits measured.

### Procedure

About 700 experimental plants (John Baer) were set out in the field and 200 were left in the greenhouse, in 8-inch pots. These were all pruned to one stem and staked; and as new buds appeared in the leaf axils they were removed to keep the plant in the one-stem condition. Next to the experimental plants in the field were available, for comparison, other John Baer plants grown in the usual way with many branches and not staked. These bore heavily during the early part of the season. Beginning with the third cluster all fruits set were allowed to develop on the control plants. The experimental plants, on the other hand, were permitted to bear fruits in only one cluster per plant, and not more than eight fruits per cluster. At the time of blossoming 117 plants were selected for bearing in the third cluster, 120 for the fourth, 124 for the fifth, and 97 plants bore fruits in the sixth cluster. Many of these plants did not develop properly and the number of plants from which the fruits were harvested was somewhat smaller than the original number of plants selected at the time of blossoming. Attempts were made to select uniform plants which had unbranched flower clusters; but this was not entirely feasible for the fifth and sixth clusters.

The growing conditions were very satisfactory until about the middle of July when the temperature became quite high and remained so for several weeks. There was also very little rain during this time but the latter con-

<sup>1</sup> Paper from the Department of Botany and Botanical Gardens of the University of Michigan, no. 636.

dition was remedied by one thorough watering of the ground in which the plants grew. The flowers in the fifth and sixth clusters were, for the most part, produced during the hot, dry weather. The flowers and the fruits produced were, however, normal in appearance, though it is impossible to say what effect this weather condition may have had upon the size of the ovary and later upon the fruit.

As the flowers opened in the different clusters, the first five flowers in each cluster of the experimental plants were picked and the ovaries preserved. The ovaries were sectioned longitudinally and length determined. These flowers of course were picked not from the plants that were permitted to set fruits in this cluster, but from a different group of plants. In the control culture, where there were no extra plants, the first flower in a cluster was removed from 20 plants, likewise the second flower in the cluster was removed from 20 other plants, and similarly flowers were removed from the third, fourth, and fifth position in the cluster from other plants. In this way only *one* flower was removed from any one cluster. The ovaries from these flowers were sectioned and measured as in the experimental plants. When mature, the fruits were picked, and the diameter and length measured. The fruits were also cut crosswise to count the number of locules. From the first 5 fruits in each cluster, from 20 plants of each lot of the experimental plants, and from all of the fruits of 20 control plants, sections for cell-size study were removed and preserved. These sections were always taken from the same part of the fruit, namely the equatorial region, and only the ovary wall or pericarp was used. After all the fruits had been removed from these plants the stems were cut a few centimeters below and above the insertion of the fruiting cluster. The pieces of stems, properly labeled, were taken into the laboratory where hand sections were made four to five centimeters below the cluster, and the pith diameter measured with a microprojector.

The volume of the ovaries and fruits was obtained by multiplying the squared diameter by the length. This gave the volume of a cube having the dimensions of the fruits. Inasmuch as the correction factor for the volume of a sphere would be the same for all and as we were interested only in relative values we did not make this correction.<sup>2</sup>

For the cell size determination, hand sections stained with Delafield's hematoxylin were used. HOUGHTALING (3) has found that these cells are approximately spherical. The cells from the ovaries were measured by the aid of a micrometer eye-piece and high magnification. The cells from the mature fruits were projected, traced on paper and the longest diameter and the diameter at right angles to it were measured. Ten cells from each fruit

<sup>2</sup> If the fruit is considered to be an oblate spheroid the approximate volume would be obtained by multiplying our values by  $\pi/8$ .

were measured. This made 50 cells from each cluster, and as 20 plants were used, 1000 cells were measured for each level on the experimental plants. That is, 1000 cells were measured from the fruits growing in the third cluster, 1000 from the fourth, etc.; in all, 4000 cells were measured from the fruits of the experimental plants. The average number of fruits per cluster of the control plants was approximately 2.3; the total number of cells measured per cluster of the control plants therefore was about 460, or a total of 1840 cells from the control plants.

### Results

The first question to be answered was: what is the influence of increase in size of the plant upon the size of the ovary? As mentioned in the section

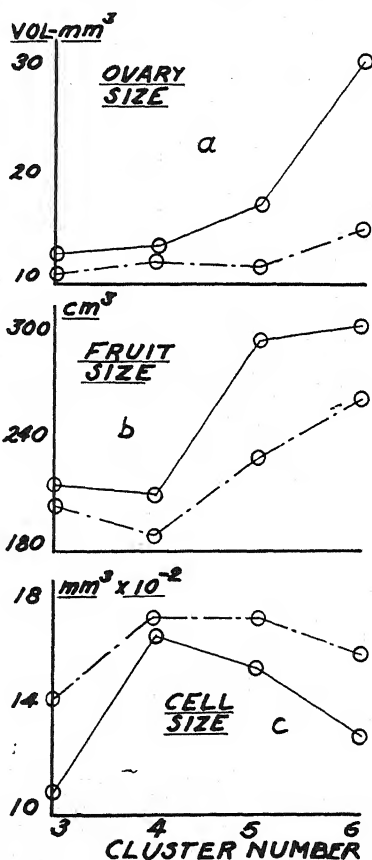


FIG. 1. Solid lines denote the experimental plants, interrupted lines the control plants. Graph a, average volume of the ovaries of the first five flowers from the different clusters; graph b, average volume of mature fruits; graph c, average volume of the cells from mature fruits from different clusters.

on procedure, the experimental plants produced fruits only in the third, fourth, fifth, or sixth cluster, *i.e.*, in one cluster per plant. It is obvious that the plants that bore fruits in the fifth cluster were much larger, and presumably had stored more food when the flowers were being produced, than those plants that bore fruits in the third cluster. Figure 1a shows that in the experimental plants the older and larger plants, *i.e.*, those plants that bore fruits in the fifth and sixth clusters, produced ovaries that were larger than those produced by plants that were younger and smaller. There was, however, no constant relation between plant size and ovary size. In the control plants, which were supplying food to growing fruits at the same time that the ovaries were forming, the ovary size was only very slightly larger in successive clusters. Evidently the nutritional level of the plant at the time the flower bud develops greatly influences the size of the ovary formed in the bud.

Figure 1b gives some very interesting information concerning the size of the fruits developed under the different nutritional levels. The larger ovaries in the fifth and sixth clusters of the experimental plants did not grow into correspondingly larger fruits. They were larger than those produced from smaller ovaries but not proportionately to the difference in the original ovaries. This is especially true of the sixth cluster, where the volume of the ovaries of the control plants was only 57 per cent. of the volume of the ovaries of the experimental plants, while the mature fruits were 87 per cent. as large as the corresponding fruits in the experimental plants. The volume of the ovaries of the control plants in cluster three was 81 per cent. of that of the ovaries of the experimental plants, while the mature fruits were 95 per cent. as large. The fruits from the control plants are thus seen to have gained on the fruits from the experimental plants during their development. Obviously this was brought about either by the production of more new cells in the control fruits than in the experimental fruits during the period between flowering and fruit maturation, or the cells were larger in the control fruits. It is possible that both of these conditions occurred together. HOUGHTALING (3) has shown, however, that little or no cell division occurs in the tomato after the setting of the fruit, and this has been verified in the present investigation. That leaves the matter of difference in cell size. Figure 1c shows the size of the cells in the fruits from the different clusters in the two groups of plants. The control fruits have larger cells throughout than the corresponding experimental fruits. Is this difference sufficient to account for the greater size in the fruits of the control plants? Table I shows the percentage relationship between the volume of the ovaries, fruits, and cells of the control and experimental plants. In cluster three the fruits from the controls gained 14 per cent. on the experimental fruits, while the cell size of the former was 32 per cent. greater than

TABLE I

PERCENTAGE OF THE VOLUME OF THE OVARIES, FRUITS, AND CELLS OF THE CONTROL IN TERMS OF THE EXPERIMENTAL PLANTS

CLUSTER NUMBER	OVARIES*	FRUITS*	CELLS*
	%	%	%
3 .....	81	95	132
4 .....	94	91	105
5 .....	68	78	114
6 .....	57	87	124

\* Values obtained by dividing those of the control plants by the corresponding values for the experimental plants.

that of the latter. This is a rather large discrepancy. In the remaining clusters the difference is not so great.

If we grant that the greater increase in the control fruits is entirely attributable to the larger cells, there is still left the problem of accounting for these larger cells. GUSTAFSON and STOLDT (2) found that when the leaf area per fruit was increased, the volume of the fruits increased as much as 40 per cent. In these experiments we have increased the size of the ovaries as much as 73 per cent. (in cluster 6) by preventing the plants from setting fruits, yet fruits from these ovaries were only 14 per cent. larger than the control fruits grown from the much smaller ovaries. There seems to be a limit to the size of a fruit that a plant can produce; beyond that it cannot grow.

The large fruits were very irregular in shape, while the smaller ones were regular and symmetrical. To determine the cause of this irregularity we began to cut the fruits crosswise and noticed that the larger fruits were composed of more carpels, *i.e.*, had more locules, and that the carpels were very crowded and, due to the crowding, often misshapen. This find led us to count the number of locules per fruit. When all of the fruits studied were analyzed it was found that there was a positive correlation of  $0.8421 \pm 0.0431$  between the number of locules and the fruit size.

A comparison between the size of the fruits produced by the plants pruned to one stem and those grown on plants with a number of stems, and bearing heavily all summer long, is very interesting. From the latter group 100 ripe fruits were collected at the time the fruits were collected from the sixth cluster of the experimental and control plants. While the position of these fruits on the stem was not determined, they were probably from the fifth or sixth cluster. A comparison between the size of these fruits and that of the fruits from the sixth cluster of the control and experimental plants will therefore not be far wrong. These values are: for the experimental fruits, average volume 303 ml., for the control 265 ml. and for the

fruits from the normally grown plants only 154 ml. The fruits from the experimental plants were nearly twice as large as those from the ordinary plants. This again brings out the fact that one can increase the size of a fruit, though evidently not indefinitely. Another comparison which can be made is that between the cell sizes of these classes of fruits. The average size of cells from the fruits from the sixth cluster of the experimental plants was  $13.161 \text{ mm.}^3$ , from the control fruits,  $16.255 \text{ mm.}^3$ , and from the normally grown fruits,  $10.304 \text{ mm.}^3$ .

We were also interested in the relation between the size of the ovary and its position in the fruiting cluster. It has been generally recognized that the fruits first produced in a cluster are the largest (GUSTAFSON, 1). Figure 2 shows that the same is also true for the size of the ovary in control

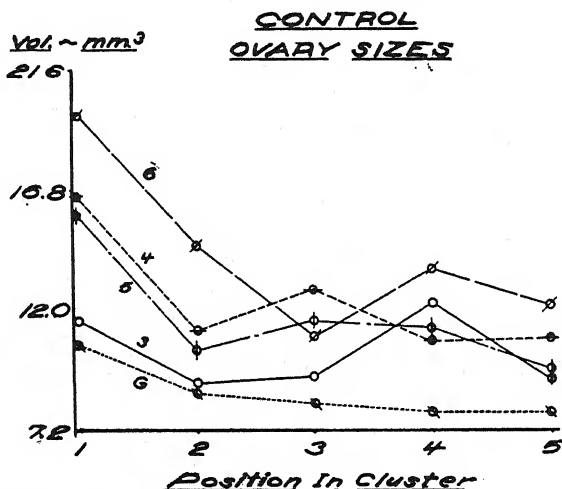


FIG. 2. Volume of the ovaries in the different positions in a cluster. The four clusters studied in the controls are represented. Curve G, volumes of the ovaries from the fifth and sixth clusters of the greenhouse grown plants.

plants. This is not always true, however, when the plants have a high nutritional level during the development of the flower buds (fig. 3). In cluster three the first and the fifth ovaries were of the same size, and in the fifth cluster the fifth ovary was much larger than the first. In the sixth cluster, which flowered during the hot dry weather, there was a very large difference in size between the first and the second ovary. The experimental plants produced more flowers in the sixth cluster than they had in the other clusters. Whether the production of this large number of flowers was a sufficient drain upon the food supply entering the fruiting branch to decrease so sharply the size of the ovaries, or whether the hot and dry weather was the cause, we do not know. It should be noted that even though the fifth ovary

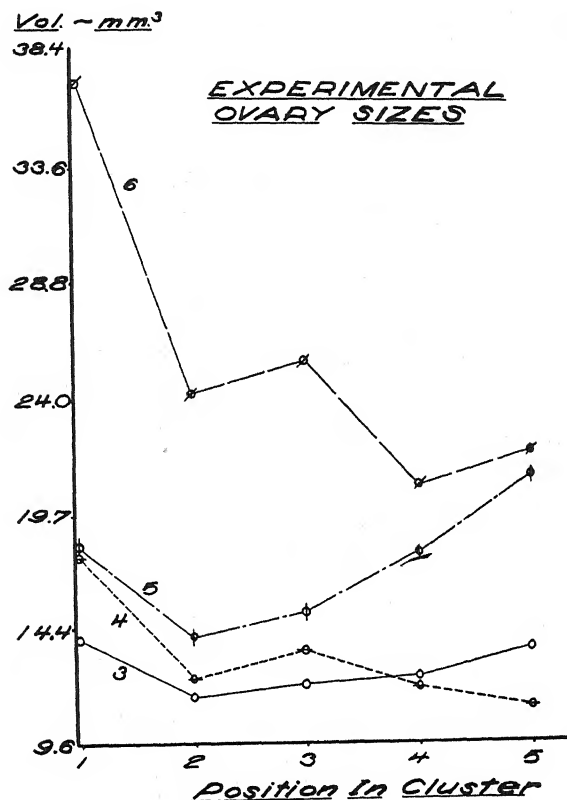


FIG. 3. Same as figure 2, but representing the experimental plants. Unlike the ovaries from the control plants there is not always a decrease here in size in successive ovaries in a cluster.

in cluster six is much smaller than the first one it still is larger than the first ovary in the control plant for the same cluster.

As mentioned above, it is usually stated that there is a decrease in the size of the fruit with the order of development in a cluster. This is borne out by figure 4. The results in the experimental fruits (fig. 5) are somewhat variable. Cluster 3 is very much like the corresponding one in the control plants, and there is no reason why it should be different, as it is the first bearing cluster in both groups. Clusters 4 and 5 are very different in the two groups. In cluster 5 of the experimental plants the sixth fruit is 68 per cent. larger than the fruit in position one; in the corresponding cluster in the control the fifth fruit (which is the last one) is only 52 per cent. as large as the fruit in position one. In the sixth cluster of the experimental plants the fruits, like the ovaries, are decidedly peculiar. The sixth fruit is smaller than the first, but not so much smaller as was the ovary.



The results for the sixth cluster in the experimental plants are based on a smaller number of plants than any of the others; and some of the fruits last produced may not have been fully grown, as they had to be picked before they were completely ripe. This, together with the fact that the later ovaries were much smaller than the first one, may account for the smaller size of the fruits later produced in cluster 6 of the experimental plants.

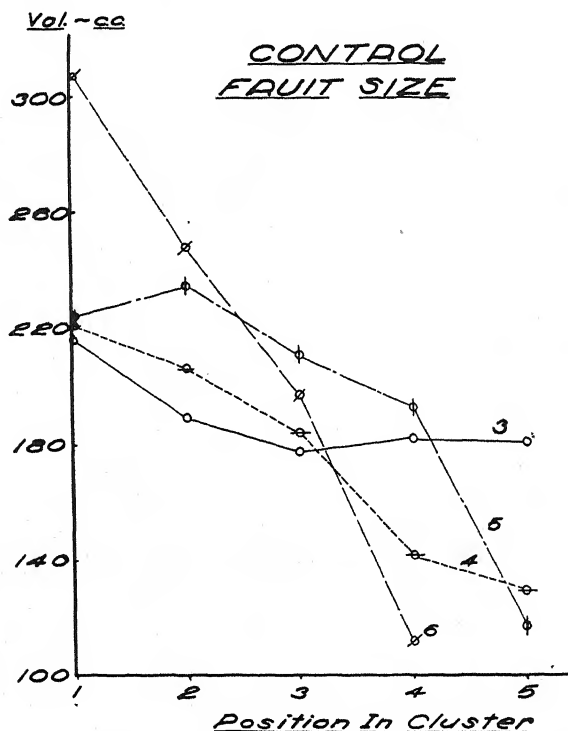


FIG. 4. Volume of the fruits in the different positions in a cluster. The four clusters studied in the control are represented.

SINNOTT (4) from his work on the bean and on *Acer saccharum* came to the conclusion that there is no correlation between the size of the body of a plant and the organs produced by it, such as leaves and fruits, but that there is a correlation between the size of the growing point and the body produced by it. It was impractical to measure the size of the growing point so he conceived the idea of measuring the diameter of the pith of the stem near the point where the organ was located. He considered that, as the pith was laid down by the growing point, it bore a certain relation to the size of the growing point. He measured the leaves of *A. saccharum* and the diameter of the pith in the internode below the attachment of a leaf and found a

correlation between the size of the pith and the size of the leaves coming from the stem nearby.

Having the fruit volume already, we thought it worth while to determine whether in the tomato there was also a correlation between pith size and size of the organ (fruit) produced by the stem. We therefore measured the diameter of the pith of 20 stems for each group of experimental plants; and the pith of 20 control plants was measured below each cluster of fruits. The greenhouse plants were also used. The pith diameter was measured from hand sections from the internode below the fruits. There was found to be

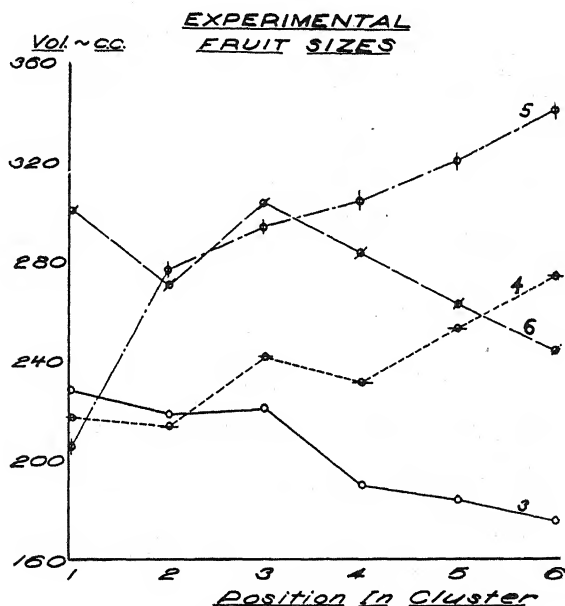


FIG. 5. Same as figure 4, but representing the experimental plants.

no correlation for the individual groups, but when the three groups were brought together there was a positive correlation of  $0.6230 \pm 0.0505$  between pith diameter and fruit volume.

### Discussion

These experiments show beyond a doubt that the size of the fruits can be increased considerably by increasing the size of the vegetative structure before the time at which the flowers are produced, but it is equally obvious that at least in the John Baer tomato the size cannot be increased indefinitely. If the ovaries are increased beyond the normal, then the cells do not enlarge as much as they do in fruits that are developing from smaller ovaries.

How are we to explain this? SINNOTT would say that the growing point was larger at the time the fruit buds were initiated and the larger meristem produced larger ovaries. Unfortunately we did not measure the size of the ovaries from the same plants in which we measured the pith diameter; and we have the pith diameters for only 20 plants for each cluster in the experimental plants and 20 plants all told for the control plants. The data for the pith diameters and the size of the fruits produced in the clusters just above are given in table II. The table shows that in the control, in which

TABLE II

AVERAGE VOLUME OF THE FRUITS PRODUCED IN A CLUSTER AND THE PITH DIAMETER OF THE INTERNODE JUST BELOW

NO. OF CLUSTER	EXPERIMENTAL FRUITS		CONTROLS	
	DIAMETER OF PITH	SIZE OF FRUIT	DIAMETER OF PITH	SIZE OF FRUIT
	<i>mm.</i>	<i>ml.</i>	<i>mm.</i>	<i>ml.</i>
3 .....	7.0	226.2	7.2	214.2
4 .....	6.9	216.5	6.2	213.9
5 .....	6.4	283.9	5.1	244.7
6 .....	7.3	289.5	5.3	328.4

the diameter of the pith below successive clusters of the same 20 plants was measured, the pith diameter decreased upward so that the diameter below the third cluster was much larger than that below the sixth; yet when we refer to figure 1*a* we note that the ovaries from the sixth cluster were somewhat larger than from the third. The size of the mature fruits was much larger from the sixth cluster, with the smaller pith, than from the third cluster, below which the pith was the largest. The correlation between pith size and fruit size for the control is  $-0.0164 \pm 0.1103$ . There is less variation in the size of the pith from the different levels in the experimental plants. Again referring to figure 1*a* we note the very much larger ovaries produced by the experimental plants in the sixth cluster than in the third, though the pith of the mature plants was only slightly larger below the sixth cluster. The correlation between pith size and fruit size in the experimental plants was only  $0.3248 \pm 0.1023$ . In both the control and experimental plants the correlation between pith diameter of the stem and the average size of the fruits produced at that point of the stem is not significant.

Another matter to be discussed is that of the cell size. Why do the cells grow larger in the smaller ovaries of the control fruits, so that the resulting fruits are nearer to the size of the experimental fruits than one would expect them to be? There is here a leveling off as far as the size is concerned; the originally larger ovaries grow less than the smaller ovaries. In

an earlier paper GUSTAFSON (1) has suggested that the size of the fruit may be determined by the conduction of food and water into the enlarging fruit. The vascular system within the fruit of the John Baer tomato is very poorly developed and if the demand upon it becomes too great it is likely that its capacity for conduction may not be sufficient for the need of the fruit and the results will be small cells if there are many of them in a fruit. It seems to us, therefore, that the poor distribution of food materials in the fruit tends to keep the fruit down to a maximum size beyond which they cannot grow. If the ovaries contain many cells, these cells do not grow as large in the maturing fruits as they do in fruits having fewer cells in the ovary stage.

### Summary

1. In these experiments 4 lots of John Baer tomato plants have been used: (a) plants grown in the greenhouse; (b) plants grown in the field not pruned; (c) field plants pruned to one stem, some of which were allowed to mature all the fruits that were set, beginning with the third cluster; and (d) other plants pruned as in (c) which were allowed to bear in only one cluster per plant.

2. The field plants that were pruned to one stem produced fruits that were nearly twice as large as the fruits produced by the unpruned plants.

3. By further reducing the number of fruits produced to one cluster per plant both the ovaries and the mature fruits were greatly increased in size, but the fruits were not increased proportionately to the larger ovaries. This was caused by the fact that the cells in the fruits produced from the larger ovaries were not so large as in the fruits produced from the smaller ovaries on the control plants. One can then state that *by increasing the leaf area per fruit, the individual fruits grow much larger but there is a limit to the size of the fruit produced, dependent upon the variety.*

4. In the controls, both the ovaries and the mature fruits that were produced later in a cluster were smaller than those produced first. This was usually not true in the experimental plants.

5. There was no significant correlation between pith size of the stem and the size of the mature fruits produced at the same level on the stem, and we are of the opinion that there is a relation between the size of the plant and the fruit produced by it, *i.e.*, a large well-nourished plant bears larger fruits than does a small plant.

6. There was a significant correlation between the fruit size and the number of carpels composing it.

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## ORGANIC ACID METABOLISM OF THE BUCKWHEAT PLANT<sup>1</sup>

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### Introduction

Much of the recorded information concerning the metabolism of the organic acids in plant tissues has been obtained from measurements of diurnal changes either in the titratable acidity or in the hydrogen-ion activity of succulent plants. Such data yield little definite information with regard to the behavior of the individual acids present in the tissues. The calculation of the results of the titration of the organic acid in terms of grams or milliequivalents of any specific acid is usually based on the assumption that the predominating acid is the only one involved in the changes that may be observed. Although this is to some extent justifiable in many cases, the evidence is seldom satisfactory. BENNET-CLARK (1), in his careful review of the whole subject, points out that information on the intermediary metabolism of organic acids in plants is so fragmentary that "general pronouncements on the significance of the plant acids are at present probably of little value . . . and our knowledge of plant acid metabolism is therefore best summarized by including all the reactions which have been shown to occur in living tissues since it is usually impossible to show that such reactions do not occur in any given tissue."

Although the organic acid metabolism of plants other than succulents has been studied less extensively than that of the succulent plants, it is known that diurnal variations in pH occur in many species. Such changes have been interpreted by CLEVENGER (2), in the case of the cowpea, to signify an accumulation of organic acids during the night and utilization during the day. The changes he observed, however, are not nearly as pronounced as those often found in the Crassulaceae, and both GUSTAFSON (3) and HURD (4) have reported that the diurnal fluctuations in the pH of nonsucculents are not always associated with changes in the titratable acidity. SMALL (16) states that the variation in pH brought about by differences in the intensity of illumination of the plants is affected not only by the total concentration of acid but also by other factors connected with the metabolism of the living leaf, possibly the type of buffer system present, or the nature of the individual acids.

<sup>1</sup> The plants described in the present communication were grown at the New Jersey Agricultural Experiment Station. It is a pleasure to thank Dr. SHIVE and Dr. ROBBINS for the supply of seed, for their help and cooperation, and for the hospitality of the laboratory to one of us. A part of the expense of this investigation was borne by the Carnegie Institution of Washington.

The present study was undertaken to obtain preliminary data on the variations in the organic acids of a nonsucculent plant that shows diurnal periodicity in pH. Buckwheat (*Fagopyrum esculentum* Moench) was chosen since INGALLS and SHIVE (5) have shown that the pH of the sap expressed from both leaves and stems increases during a period of maximum illumination and decreases during the night.

### Experimentation

The seeds were germinated between moist filter papers and were transferred to a germinating net as described by SHIVE (13). On April 4, 1937, when the seedlings were about 5 cm. tall, 72 plants were selected for uniformity of size and vigor, and were transferred, in groups of six, to 12 two-quart jars filled with a nutrient solution of the following composition:  $\text{KH}_2\text{PO}_4$  0.00225 M,  $\text{Ca}(\text{NO}_3)_2$  0.0045 M, and  $\text{MgSO}_4$  0.00225 M. The nutrient solution also contained 0.5 to 1 part per million of iron and 0.25 parts per million each of boron and of manganese. The solution cultures were aerated continuously, and SHIVE's continuous-flow method of supplying fresh nutrient at the rate of about 500 to 700 ml. per day was employed. On April 28, the plants had attained a height of 55 to 60 cm. and were beginning to form flower buds. April 30, 26 days after transplantation, promised to be a bright and cloudless day so that maximal differences in light intensity during the sampling period seemed assured. Three cultures containing a total of 18 plants were therefore harvested at the start, and at each of three successive intervals during a 22-hour period. The plants were divided into leaf and stem tissue which were separately weighed and dried in a ventilated oven at 80° C. A 10-gm. aliquot of each fresh stem sample was frozen and the pH of the expressed sap was determined with a quinhydrone electrode. To conserve material for subsequent chemical analyses, pH determinations on the leaf tissue were omitted, since INGALLS and SHIVE have shown that the pH changes in the blade are similar in magnitude to those in the stem.

The four samples of leaf and stem tissue were obtained, one at 6:20 A.M., E.S.T., a second at 1:45 P.M., a third at 7:00 P.M., and a final one at 4:00 A.M. the following morning. The organic acid content of the dried samples was subsequently determined by specific methods (6, 7, 8, 9).

### ORGANIC ACIDS OF BUCKWHEAT

Although extensive studies on the nutrient requirements of the buckwheat plant have been carried out by SHIVE (14, 15), there are, so far as we are aware, no data available on the organic acid composition of this plant. Table I shows the organic acid content of the initial sample of tissue in percentage of the dry weight. The chief acid of this plant is oxalic, the con-

TABLE I  
ORGANIC ACID COMPOSITION OF BUCKWHEAT PLANT\*

ACIDS DETERMINED	LEAF†	STEM†
	%	%
Oxalic acid .....	11.1	3.95
Malic acid .....	1.51	1.96
Citric acid .....	0.82	0.46
Nitrate N .....	0.37	2.07
Ash .....	13.27	17.48

\* The average fresh weight of a single plant was 11.00 gm., of which 3.04 gm. were leaf, 7.19 gm. stem, and 0.765 gm. root. The average dry weight was 0.962 gm. distributed as 0.406 gm. leaf, 0.481 gm. stem, and 0.0753 gm. root.

† Data are given in percentage of the dry weight.

centration in the leaf being about three times as high as that in the stem. Malic and citric acid are also present in appreciable quantities, the former being slightly higher in the stem than in leaf tissue. Over 90 per cent. of the total organic acidity in the leaf is present as oxalic, malic, and citric acids, while in the stem only about 72 per cent. can be accounted for in terms of these three.

The stems of the plants contained a concentration of nitrate nitrogen more than five times greater than that of the leaf tissue. The quantity found is, in fact, the equivalent of nearly 15 per cent. of the dry weight, when expressed as potassium nitrate, and it is evident that a large part of the cations of the stem must be combined with nitrate ions. Accordingly a large proportion of the organic acids must be free or in the form of monobasic ions. This may account for the observation that buckwheat stem tissue, in spite of its greater ash and smaller organic acid content, is more acid in reaction than the leaf tissue.

### Results

The behavior of the organic acids of buckwheat during the day may be most simply presented in terms of the changes in actual amount of each acid during each of the three successive time periods. These were selected to give first, an interval during which the illumination increased to a maximum and the reaction of the tissues, according to INGALLS and SHIVE, might be expected to become less acid; second, a period during which the light intensity diminished and the reaction of the tissues might be expected to become more acid; and last, a period of darkness when little or no change in pH occurs.

The changes are recorded in the tables as grams or milliequivalents, and also in percentage of the total amount of each substance present at the beginning of each successive period. Table II shows the changes in water, organic solids, and ash; table III shows the changes in organic acids.



TABLE II

CHANGES IN WATER, ORGANIC SOLIDS, AND ASH OF BUCKWHEAT PLANTS DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
Period I, 6:20 A.M.-1:45 P.M.: increasing illumination and increase of pH of sap (4.20-4.40)						
	gm.	%	gm.	%	gm.	%
Water .....	+0.28	+10.63	+0.133	+ 1.98	+0.42	+ 4.50
Organic solids .....	+0.147	+41.7	+0.070	+17.6	+0.217	+29.0
Ash .....	+0.018	+33.4	+0.007	+ 8.33	+0.025	+18.1
Period II, 1:45 P.M.-7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40-4.24)						
Water .....	+0.197	+ 6.75	+0.914	+13.7	+1.11	+11.4
Organic solids .....	+0.025	+ 5.01	+0.038	+ 8.14	+0.064	+ 6.62
Ash .....	-0.002	- 2.78	-0.002	- 2.20	-0.004	- 2.45
Period III, 7:00 P.M.-4:00 A.M.: complete darkness; no change in pH of sap (4.24-4.24)						
Water .....	+0.15	+ 4.83	+0.49	+ 6.32	+0.65	+ 5.98
Organic solids .....	-0.046	- 8.78	+0.113	+22.4	+0.06	+ 5.83
Ash .....	+0.004	+ 5.72	+0.015	+16.9	+0.019	+11.95

\* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

The first period is characterized by an increase in organic acids and, accordingly, the decrease in the acidity of the tissue extract cannot be interpreted as evidence for organic acid utilization. The observed increase of 0.2 unit in pH must have some other explanation such, for example, as an increase in absorption of inorganic cations, or the conversion of nitrate to ammonia. The increase of organic acid seems to be correlated with the photosynthetic processes in the plant since, from table II, it is apparent that the largest increase of organic solids, an approximate measure of photosynthetic activity, occurred during this period.

In the whole plant, 73 per cent. of the increase of total acids is due to oxalic acid and 27 per cent. to malic, citric, and unknown acids. Although the absolute amounts of acids formed, other than oxalic, are small, the percentage increases of these acids are large. The data also indicate that the individual acids are transported from leaf to stem tissue at markedly different rates. Thus malic acid increased 42 per cent. in the stem tissue and only 32 per cent. in the blade, while the unknown acids decreased in the

TABLE III

CHANGES IN THE ORGANIC ACIDS OF BUCKWHEAT PLANT DURING THREE SUCCESSIVE TIME PERIODS IN ONE DAY

	LEAF*		STEM*		LEAF + STEM*	
Period I, 6:20 A.M.-1:45 P.M.: increasing illumination and increase of pH of sap (4.20-4.40)						
	meq.	%	meq.	%	meq.	%
Oxalic acid .....	+ 0.29	+ 28.7	+ 0.080	+ 19.3	+ 0.37	+ 25.9
Malic acid .....	+ 0.029	+ 32.2	+ 0.059	+ 41.9	+ 0.088	+ 38.1
Citric acid .....	+ 0.017	+ 34.0	+ 0.012	+ 34.3	+ 0.029	+ 33.3
Total gain of determined acids	+ 0.336	+ 29.2	+ 0.151	+ 25.6	+ 0.487	+ 27.9
Total organic acid ...	+ 0.38	+ 30.2	+ 0.13	+ 15.8	+ 0.51	+ 24.4
Unknown acids .....	+ 0.04	+ 36.4	- 0.02	- 8.70	+ 0.02	+ 5.88
Nitrate N .....	+ 0.015	+ 14.1	+ 0.074	+ 10.4	+ 0.089	+ 10.9
Period II, 1:45 P.M.-7:00 P.M.: decreasing illumination and decrease in pH of sap (4.40-4.24)						
	meq.	%	meq.	%	meq.	%
Oxalic acid .....	+ 0.04	+ 3.08	+ 0.017	+ 3.43	+ 0.05	+ 2.78
Malic acid .....	+ 0.002	+ 1.68	- 0.030	- 15.0	- 0.028	- 8.78
Citric acid .....	+ 0.006	+ 8.70	- 0.001	- 2.13	+ 0.005	+ 4.31
Total gain of determined acids	+ 0.048	+ 3.23	- 0.014	- 1.89	+ 0.027	+ 1.20
Total organic acid ...	+ 0.13	+ 7.75	+ 0.055	+ 5.76	+ 0.18	+ 6.92
Unknown acids .....	+ 0.08	+ 53.3	+ 0.06	+ 28.6	+ 0.15	+ 41.7
Nitrate N .....	- 0.020	- 16.4	+ 0.042	+ 5.35	+ 0.022	+ 2.42
Period III, 7:00 P.M.-4:00 A.M.: complete darkness; no change in pH of sap (4.24-4.24)						
	meq.	%	meq.	%	meq.	%
Oxalic acid .....	+ 0.14	+ 10.5	+ 0.058	+ 11.3	+ 0.20	+ 10.8
Malic acid .....	+ 0.019	+ 15.7	+ 0.097	+ 57.1	+ 0.116	+ 39.9
Citric acid .....	+ 0.016	+ 21.3	+ 0.013	+ 28.3	+ 0.029	+ 24.0
Total gain of determined acids	+ 0.175	+ 11.4	+ 0.168	+ 23.1	+ 0.345	+ 15.3
Total organic acid ...	+ 0.04	+ 22.6	+ 0.11	+ 10.9	+ 0.15	+ 5.40
Unknown acids .....	- 0.13	- 56.5	- 0.06	- 21.4	- 0.19	- 37.3
Nitrate N .....	- 0.010	- 9.90	+ 0.085	+ 10.3	+ 0.071	+ 7.64

\* The figures give the changes in grams per single plant in each period, and changes as percentage of the initial value in each period. The total amount of any constituent at the start of each period may be obtained by dividing the recorded quantitative change by the percentage change and multiplying by 100.

stem and increased 36 per cent. in the blade. These observations clearly show that data for the total organic acids alone cannot be used to interpret the metabolic changes in the individual acids. This has also been pointed out in the case of the organic acids of the rhubarb leaf (12).

In the second period, the increase of total organic acids in the leaf tissue was only one-third of that during the first period. Synthesis of malic, citric, and oxalic acid almost ceased, and 83 per cent. of the increase in total organic acids in the leaf and stem was due to the production of acids of

the unknown group. This appears to be correlated with the fact that the plants received a diminishing amount of light and showed a corresponding diminution in photosynthetic activity. The definite loss of malic acid from the stem in this period shows that utilization of malic acid may occur when photosynthetic activity in the leaves is diminished. If the malic acid that disappeared had been transported from stem to leaf, there would have been a far more extensive increase in the leaf than was actually observed.

In spite of the utilization of malic acid in the stem, and the small increase in total acids, the tissue sap became more acid, a further indication that tissue pH is not a satisfactory index of the organic acid metabolism. Table II shows that no inorganic salt absorption occurred, and it therefore seems probable that the decrease in pH is associated with the establishment of a new equilibrium between the cations and the organic acids. The utilization of a part of the nitrate in the leaf suggests that some such rearrangement must have occurred.

The behavior of the organic acids during the period of darkness is particularly interesting. Table III shows that, although the total quantity of organic acids produced during the night by the leaf and stem was only 0.15 milliequivalent, the combined increase in oxalic, malic, and citric acid was 0.345 milliequivalent. This increase is clearly attributable to a transformation of acids of the unknown group, formed during the second period, into one or more of these three substances. In the second period, 0.15 milliequivalent of unknown acids were formed, in the third this fraction decreased by 0.19 milliequivalent. The agreement is well within the experimental error of these determinations. During the night, two distinct types of reaction appear to take place: a small quantity of organic acids is produced, and the unknown acids formed during the second period are converted into one or more of the known acids. The latter process recalls the observation that malic acid is converted into citric acid during culture of tobacco leaves in water in darkness (11). Table II shows that the buckwheat plant gains an appreciable quantity of ash during the night. It seems probable that a balance between cations and anions is thereby maintained so that no appreciable change in tissue pH occurs.

### Discussion

It is obvious that, in the buckwheat plant, diurnal fluctuation in pH cannot safely be used alone as a criterion of the changes in the organic acid composition. It would seem that only a complete study of the variations in inorganic cations and anions, as well as of the organic bases and of the individual organic acids, would yield the information necessary to explain satisfactorily the observed fluctuations in reaction of the tissue juices. No such comprehensive set of data has, to our knowledge, ever been assembled

for a plant tissue. In a recent paper (10), we have shown that a definite correlation exists, in the case of samples of tobacco leaf for which relatively complete ash analyses were available, between the excess of positive ions in the ash and the quantities of organic acids present in the tissue. The organic acids obviously play a major rôle in the physico-chemical relationships upon which the hydrogen-ion activity and buffer capacity of the cell fluids depend. The present results with buckwheat clearly indicate that these relationships are far from simple, and hold out little promise that they can be accounted for from such limited data as observations of the pH or of the titratable acidity.

### Summary

The buckwheat plant contains oxalic acid as the chief organic acid of both leaf and stem. Malic acid is present in relatively smaller amounts and citric acid is present in minor proportions. These three acids make up about 90 per cent. of the organic acids of the leaf and about 72 per cent. of the organic acids of the stem. The leaves of one plant contain about 1.5 times as much total acid as the stem.

During the period of maximal illumination early in the day, photosynthesis is most active and the organic acids increase. Nevertheless there is a decrease in hydrogen-ion activity of the sap of the stem. In the later part of the day, malic acid is utilized and there is an increase in the group of unknown organic acids. The net change in total organic acids is a small increase, but the hydrogen-ion activity of the sap of the stem increases to approximately the early morning level. Photosynthesis is much less active in this period, and the assimilation of inorganic ions from the culture solution is also depressed.

During the night, there is a small increase in total organic acids and a marked interconversion of acids of the unknown group into one or more of the known acids takes place. There is no change in hydrogen-ion activity in the sap of the stem.

These observations show that interpretations of the changes in the hydrogen-ion activity of the sap of the plant in terms of an increase or decrease in organic acids cannot be made in the absence of specific information regarding these substances. It is obvious that such factors as absorption of inorganic ions by the roots, and the distribution of the inorganic and organic basic constituents within the tissues, as well as photosynthesis, play an important rôle in the changes in acidity that occur.

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# DETERMINATION OF AMMONIA AND AMIDE NITROGEN IN CONNECTION WITH THE CHLORATE METHOD FOR NITROGEN IN PLANT TISSUES<sup>1</sup>

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## Introduction

In previous work with the chlorate method (1, 2) for determining nitrogen in plant tissues, materials were used in which the amounts of ammonia and amide nitrogen were either absent or negligible in relation to the total nitrogen present.

In working with samples containing significant amounts of ammonia compounds or amide nitrogen, it is necessary to accompany the determination with a Nessler test for ammonia. The chlorate oxidation changed all nitrogen in the plants analyzed to nitrate nitrogen with the exception of nitrogen already in the ammonia form or nitrogen in compounds which readily hydrolyze to ammonia such as amides.

In some studies of nitrogen in plants this may be a decided advantage since this divides the nitrogen into two important groups:

1. Nitrates, alkaloids, proteins, and amino acids the nitrogen in which is oxidized to nitric acid and determined by phenoldisulphonic acid, and

2. Ammonia, amides, and other readily hydrolyzable nitrogen compounds the nitrogen in which is converted to ammonia and determined by the Nessler test. These are the usual compounds to be expected in plants. Of course small amounts of the rarer nitrogen compounds may be present, but it is pretty certain in the chlorate oxidation that all nitrogen in plants is either oxidized to nitric acid or caught as ammonium sulphate. The same is probably true for all nitrogen compounds in general. It will be shown later that the N in pyridine which is not determined by the regular Kjeldahl procedure was determined by the chlorate method.

In the soluble nitrogen test (1) on plant extracts from the more mature conducting tissue of lower petioles of rapidly growing long-day plants, most of the nitrogen is in the form of amino acids or nitrates and for practical use to determine nitrogen deficiencies the Nessler test may be omitted, but for detailed work it should be included. MURNEEK (3) shows that short-day plants contain considerable amide and  $\text{NH}_3$  nitrogen. Inclusion of the Nessler test enables a more detailed study to be made, since, after treatment with  $\text{H}_2\text{SO}_4$  and  $\text{NaClO}_3$ , the phenoldisulphonic test shows all active nitrogen (amino acids and nitrates) present which is in a form for immediate use in the anabolic processes, while the Nessler test shows the reserve nitrogen

<sup>1</sup> The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

(amides, especially that in asparagine and other compounds readily hydrolyzed to ammonia). A third group would be free ammonia found by making a direct test on the original extract, but this form is not usually found except in very small amounts in normal plant extracts.

### Procedures

**AMMONIA AND AMIDE NITROGEN.**—Ammonia nitrogen is determined in an aliquot of the same solution as is used in determining nitrate, alkaloid, protein and amino nitrogen in the chlorate oxidation (1, 2).<sup>2</sup> If a large amount of amide nitrogen is suspected the solution should be refluxed considerably longer (about one-half hour longer) than is directed in the regular chlorate procedure (3 to 5 minutes) to insure complete hydrolysis of amides although as a rule this is unnecessary in plant samples. This may be tested by Nesslerizing with and without extra refluxing.

Put an aliquot such that between 0.05 and 0.30 mg. of  $\text{NH}_3$  are present into a 200-ml. Erlenmeyer flask. Usually 1 to 5 ml. are sufficient. Add almost 20 ml. of water and a small piece of litmus paper. Add strong ammonia-free NaOH until the paper just turns blue. Now add normal hydrochloric acid until the paper just turns red. Remove the paper and cool the solution to about room temperature under a tap. Make the volume to about 90 ml. with water. Add 3 ml. of Rochelle salt reagent (4) and 3 ml.

<sup>2</sup> Put 100 to 300 mg. of dry tissue, or 500 to 1500 mg. of green tissue, into a 200-ml. Erlenmeyer flask. There should be at least 0.5 mg. of nitrogen in the sample used. Add 2 ml. of 50 per cent. sodium chlorate solution for each 100 mg. of dry sample, or each 500 mg. of green sample. Mix well with the sample and allow to soak a few seconds. Add 25 ml. of 52 per cent. by volume sulphuric acid and attach the flask to a water-cooled reflux condenser. Arrange the connections to the condenser in such a manner that the flask can be shaken readily. Heat rather slowly at first. When the reaction begins to become active, shake to wash down particles from the sides. Do not allow blackening of particles on the sides of the flask if it can be helped. However, if the particles of samples have been soaked with a 50 per cent. chlorate solution some blackening does no harm. Heat until the sample is completely gone (2 to 3 minutes). Stop the heating while there is still a very faint yellow chlorine color. Flush out the condenser with two successive 5-ml. portions of 52 per cent.  $\text{H}_2\text{SO}_4$ , and cool the flask in a cold water bath. After detaching, cool to room temperature.

Make the cooled solution up to a convenient volume. If the quantity of nitrogen present is small, the volume should be as small as possible. Immediately put exactly 1 or 2 ml. (use an accurately calibrated pipette) of the solution into a 25-ml. test-tube and add 3 or 6 ml. of phenoldisulphonic acid, mix well, and allow to stand about one-half minute. (If a very reddish solution forms here, the heating was not continued long enough to decompose excess chloric acid. The solution should remain almost colorless.) Wash into a 200-ml. Erlenmeyer with about 10 or 20 ml. of water and make alkaline with 40 per cent. NaOH solution, adding it until the maximum yellow color is produced and a small excess of alkali is present. Make to a volume which brings the yellow color near to the standard. After thorough mixing, the clear solution is compared with a standard (1 ml. = 0.0025 mg. N) in a colorimeter.

of Nessler reagent (4). Make to exactly 100 ml. and compare with a standard (4) containing 0.001 or 0.002 mg. of  $\text{NH}_3$  per ml. The solution should be read within one-half hour.

**TOTAL NITROGEN.**—Convert the  $\text{NH}_3$  found to N as  $\text{NH}_3$  by multiplying by 14/17. Add this N to the nitrate nitrogen found by the use of phenol-disulphonic acid (1, 2). This sum gives the total nitrogen in the sample.

**TOTAL SOLUBLE NITROGEN IN PLANT EXTRACTS.**—Oxidize 0.5 to 1.0 ml. of the plant extract by chlorate and fuming sulphuric acid as directed in the rapid soluble nitrogen test (1). After the fumes have been blown out by means of a bent glass tube until the solution is no longer yellow, wash the solution into an Erlenmeyer flask with 20 ml. of water and reflux at least one-half hour if much amide N is thought to be present. This is not necessary in many plant extracts where only small amounts of amides are present. A test with and without refluxing will determine this. Cool and treat the solution exactly as in the above directions for ammonia nitrogen.

If considerable plant extract is available the nitrogen may be determined as for total nitrogen in solids. Place 5 to 20 ml. of extract (depending on the N present) in a 200-ml. Erlenmeyer flask with 1 gm. of sodium chlorate. Attach to a reflux condenser, pour a volume of concentrated sulphuric acid (95 per cent.) equal to that of the extract used through the top of the condenser and immediately heat with a strong flame. If much amide nitrogen is suspected, heat for one-half hour after the yellow chlorine color disappears. Proceed for nitrate and ammonia nitrogen as previously directed.

**NITROGEN BY DIGESTION WITH SULPHURIC ACID AND DIRECT NESSLERIZATION.**—An aliquot, usually 10 ml., is digested with 10 ml. of concentrated sulphuric acid until colorless. The volume is then made to exactly 10 ml. with 50 per cent. sulphuric acid. An aliquot is then taken (0.5 to 2 ml. usually) and 20 ml. of water added. Litmus paper is added and the solution neutralized and Nesslerized as directed under procedure for ammonia nitrogen. This does away with distillation and titration as in the regular Kjeldahl procedure and lessens danger of losses. It also enables smaller amounts of N to be determined accurately. Note the following precautions:

(a). The appearance of cloudiness in the Nessler solution may be caused by several things:

- (1) Solution too alkaline
- (2) Too much  $\text{NH}_3$  present
- (3) Presence of too large amounts of sulphate
- (4) Long standing
- (5) Heat

Cloudy solutions must never be used for comparison in accurate work, although fairly accurate results may be secured in slightly cloudy solutions if the standard and unknown are made up under the same conditions and at



the same time. If proper precautions are taken and directions are followed clear solution will result. If extremely small amounts of  $\text{NH}_3$  are being determined it is better not to use too large aliquots even if the color is light due to the introduction of too much sulphate.

(b). Of course, blanks must be run on reagents to make sure no  $\text{NH}_3$  contamination is present. Sodium hydroxide solution can be freed of  $\text{NH}_3$  by boiling.

DETERMINATION OF TOTAL NITROGEN IN PURE AMMONIA COMPOUNDS.—In order to determine whether the nitrogen in ammonium compounds could be estimated, the method was tried on ammonium sulphate, nitrate and chloride. Table I shows the results.

TABLE I  
TOTAL N IN AMMONIUM COMPOUNDS

COMPOUND	THEORETICAL PERCENTAGE	PERCENTAGE AS NITRATE N	PERCENTAGE AS AMMONIA N	TOTAL N	PERCENTAGE ERROR
$(\text{NH}_4)_2\text{SO}_4$ ...	21.20	1.56	19.33	20.89	-1.46
$(\text{NH}_4)_2\text{SO}_4$ ...	21.20	1.25	20.58	21.83	+2.97
$\text{NH}_4\text{NO}_3$ .....	35.00	17.85	17.52	35.37	+1.11
$\text{NH}_4\text{NO}_3$ .....	35.00	17.60	17.42	35.02	+0.11
$\text{NH}_4\text{Cl}$ .....	26.17	6.00	20.58	26.58	+1.57
$\text{NH}_4\text{Cl}$ .....	26.17	4.81	21.82	26.63	+1.76

Fifty-milligram samples of the pure salt were oxidized as directed in the chlorate method (1, 2), the nitric acid determined by phenoldisulphonic acid and ammonia determined by Nessler's reagent as directed in the preceding procedures.

The results, except one, are slightly higher than theoretical. This shows that all the nitrogen is accounted for. In the case of  $\text{NH}_4\text{NO}_3$  all nitrate was recovered and very little  $\text{NH}_3$  oxidized.

It will be noted that a small amount of ammonia was oxidized in pure solutions. This was especially true with ammonium chloride. In order to see if this occurred in the presence of organic matter, sugar was added with the 50 mg. of  $\text{NH}_4\text{Cl}$ . The results were as follows:

SUGAR ADDED MG.	THEORETICAL PERCENTAGE OF N	PERCENTAGE OF N AS $\text{NO}_3$	PERCENTAGE OF N AS $\text{NH}_3$	TOTAL N DETERMINED
50 .....	26.17	5.50	21.11	26.61
100 .....	26.17	0.18	25.74	25.92
200 .....	26.17	0	26.35	26.35
200 .....	26.17	0	26.35	26.35

These data show that in the presence of sufficient organic matter no ammonia nitrogen is oxidized and hence the separation of nitrogen into

ammonia and other nitrogen is quantitative when enough organic matter is present.

DETERMINATION OF TOTAL NITROGEN IN ASPARAGINE.—Pure asparagine was tested to see what happened to the nitrogen in the chlorate oxidation. The results are shown in table II.

TABLE II  
TOTAL N IN ASPARAGINE

COMPOUNDS	PERCENTAGE OF N AS NO <sub>3</sub>	PERCENTAGE OF N AS NH <sub>3</sub>	PERCENTAGE TOTAL N	THEORETICAL PERCENTAGE OF N
50 mg. asparagine	10.59	10.62	21.21	21.20
50 mg. asparagine	10.71	10.63	21.34	21.20
50 mg. asparagine				
+ 50 mg. sugar ...	10.59	10.62	21.21	21.20
50 mg. asparagine				
+ 50 mg. sugar ...	10.75	10.53	21.28	21.20
50 mg. asparagine		Incomplete		
+ 100 mg. sugar		oxidation		
50 mg. asparagine				
+ 10 ml. H <sub>2</sub> O.				
Acid through condenser .....	10.55	Not determined		

The data of table II show that a quantitative separation of the amino and amide nitrogen in asparagine was made and that all nitrogen was accounted for. Too much sugar resulted in incomplete oxidation.

This gives definite proof that the method may be used in grouping nitrogen compounds as previously discussed.

TABLE III  
N IN PLANT EXTRACTS (MILLIGRAMS)

EXTRACT USED	N AS NO <sub>3</sub> BY CHLORATE METHOD (1)	N AS NH <sub>3</sub> BY CHLORATE METHOD (1)	SUM OF N AS NO <sub>3</sub> AND NH <sub>3</sub>	TOTAL NITROGEN BY SULPHURIC ACID DIGESTION	
				DIRECT NESSLERIZATION	DISTILLED* AND TITRATED
	mg.	mg.	mg.	mg.	mg.
10 ml. tomato ...	0.016	0.0413	0.0573	0.0410	.....
10 ml. " .....	0.016	0.0400	0.0560	0.0395	.....
10 ml. bean .....	2.70	2.45	5.15	4.88	4.89
10 ml. " .....	2.68	2.47	5.15	4.88	4.53
10 ml. bean† .....	2.63	2.52	5.15		

\* Distillation and titration by HOWELL D. SPEARS of the Feed Control Department.

† Only 0.5 ml. was used in this case and the test was made by the rapid soluble N test (1).

COMPARISON WITH THE KJELDAHL METHOD.—In order to further check the method, nitrate-free tomato and bean tissues were secured by growing the plants in pure white sand for two weeks. The tissue was extracted with 2 per cent. acetic acid by adding a small amount of charcoal and grinding 3-gm. samples with 10 ml. of 2 per cent. acetic. The extracts were poured together until 50 ml. were obtained. The nitrogen was then determined by 4 different methods. The results are presented in table III.

The small amount of nitrogen in the tomato extract was due to true N deficiency. The quantity in 10 ml. of extract was too small to be estimated by the usual Kjeldahl procedure. The N in the bean extract came from the seed since the beans were used in the seedling stage. No nitrate nitrogen would be expected from the seed. Small amounts of readily oxidized nitrogen must have been present, however, since the Kjeldahl results are slightly low in the beans and considerably lower in the tomato. The total chlorate method and soluble methods check exactly, although the nitrate and ammonia nitrogen were slightly different. These results show that the chlorate methods gave very accurate results on the plant extracts tested. These methods seem to be especially advantageous where nitrate nitrogen is present and where a division into the major groups of nitrogen compounds is desired.

SOLUBLE NITROGEN TEST.—Tests were made on several compounds to see what part of the nitrogen was oxidized to nitrate by the soluble N test. Table IV gives the results.

TABLE IV  
N BY SOLUBLE N TEST

COMPOUND USED	Mg. N ADDED	Mg. N AS NO <sub>3</sub> FOUND	COMPOUND USED	Mg. N ADDED	Mg. N RECOVERED
Sodium nitrate ...	0.05	0.0498	Aspartic acid	0.025	0.0254
Picric acid	0.05	0.0500	“	0.025	0.0250
Ammonium sulphate	0.05	0.0	“	0.025	0.0252
Asparagine	0.05	0.0250	“	0.025	0.0254
“	0.05	0.0250	“	0.025	0.0249
“	0.05	0.0244	“	0.025	0.02496
“	0.25	0.1250			
“	0.25	0.1255			
“	0.25	0.1248			
“	0.10	0.0500			

The data of table IV show that all nitrate, nitro, and amino acid nitrogen was recovered quantitatively, while ammonia and amide nitrogen was not obtained at all.

Tests were then made to see if Nessler tests would account for all the nitrogen. Table V shows these results.

TABLE V  
INCLUSION OF NESSLER TEST IN SOLUBLE N TEST

COMPOUND USED	Mg. N ADDED	Mg. N AS NO <sub>3</sub>	Mg. N AS NH <sub>3</sub>	TOTAL
Asparagine .....	0.05	0.025	0.02495	0.04995 (refluxed)
“ .....	0.05	0.025	0.0106	0.0356 (not “ )
“ .....	0.05	0.025	0.02514	0.05014 (refluxed 10 minutes)
“ .....	0.05	0.025	0.0058	0.0308 (not refluxed)
Asparagine + 5 mg. sugar .....	0.05	0.025	0.0052	0.0302 ( “ “ )
Asparagine + 5 mg. sugar .....	0.05	0.025	0.02514	0.05014 (refluxed 20 minutes)
Ammonium chloride	0.05	0.0	0.05	0.050
“ “	0.10	0.0	0.105	0.105

The results in table V show that amide nitrogen is recovered if the solution is refluxed and Nesslerized.

The soluble nitrogen test was further checked against the total chlorate method on N in tomato extract, with results shown in table VI.

TABLE VI  
N BY TOTAL CHLORATE AND SOLUBLE TEST

EXTRACT	N AS NO <sub>3</sub>	N AS NH <sub>3</sub>	METHOD
	<i>p.p.m.</i>	<i>p.p.m.</i>	
Field tomatoes stopped by cold in fall	1110 1020	1650 1694	Total chlorate Soluble
Greenhouse plants	1040 800	1875 1852	Total chlorate Soluble

The methods checked almost exactly with the exception of nitrate N in the greenhouse plants. No explanation of why these did not check can be offered. The exact checks between these two methods in table III and the close checks with the Kjeldahl, however, offer proof that this was an experimental error of some kind and that the soluble N test is accurate. It was found that when rather large amounts of nitrogen are present, care must be taken to cool the solution before adding phenoldisulphonic acid and that it is best to double the amount of phenoldisulphonic acid added.

TOTAL NITROGEN BY COMPLETE CONVERSION TO NITRIC ACID.—The sample is treated as in the regular chlorate oxidation (1, 2) to the point where the yellow color begins to get light. Instead of stopping the refluxing then, another addition of about 0.5 gm. of sodium chlorate is made through the condenser and vigorous refluxing is continued. It is not necessary to remove

the flame while adding chlorate since the high temperature prevents  $\text{ClO}_2$  formation. It is best not to let the solution cool before adding the chlorate. The chlorate addition is repeated whenever the yellow color becomes faint during the course of about one hour of refluxing. At the end of this time, if some chlorate is still present in the upper end of the condenser it may be necessary to flush it down with a little 50 per cent. sulphuric acid and boil until the color disappears and flush again to wash down nitric acid. The nitric acid formed is determined as in the regular procedure, by phenol-disulphonic acid.

Since, as was shown in table I, ammonium sulphate was the most difficult compound to oxidize it was tested by the above procedure. Twenty-one per cent. of N was found as nitric acid which is within 0.2 per cent. of theoretical. If some especially difficult substance is being determined an aliquot may be taken for colorimetric determination and if the conversion is thought not to be complete more chlorate can be added and the refluxing continued a while longer. If no increase is noted the oxidation is complete. If there is an increase the refluxing should be repeated until there is no more increase in the nitric acid. A record of the aliquots taken must be kept, in order to be able to compute the N content from the final readings, and added to the final figures.

**NITROGEN IN PYRIDINE.**—It has long been recognized that the Kjeldahl method will not determine the nitrogen in pyridine or quinoline. Tests were made on pyridine by the chlorate method and the following results were obtained:

Mg. PYRIDINE	Mg. N ADDED	Mg. N AS $\text{NO}_3$	Mg. N AS $\text{NH}_3$	TOTAL Mg. N FOUND
50	8.86	3.71	5.10	8.81

These results were obtained only by prolonging the oxidation period considerably, as follows:

50 mg. of pyridine and 1 gm. of sodium chlorate were placed in a 200-ml. Erlenmeyer flask and 25 ml. of 50 per cent. by volume sulphuric acid were added. The flask was attached to an efficient reflux condenser and refluxed until the yellow color was nearly gone. The heat was removed, more chlorate added (0.5 gm.) and the flask was again heated until the yellow color had nearly disappeared. This was repeated for almost 1 hour. After the yellow color had disappeared, the condenser was washed out with 10 ml. of 50 per cent.  $\text{H}_2\text{SO}_4$  and the nitrate and ammonia N determined in the regular ways. This extra refluxing is not necessary for most samples but was necessary in this case.

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## DETERMINATION OF CONSTANTS FOR CURVES OF WATER ABSORPTION BY DRY ORGANIC SUBSTANCES

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Some years ago the writers engaged in a study of water absorption by dry organic matter, such as seeds (1) and gelatin disks (2), with special reference to the temperature coefficient of water absorption by seeds (1, 3). It was found not only desirable but necessary to study the absorption data mathematically, and to attempt to refine the methods of studying the rate of water intake so that mathematical computations could replace cruder methods of measurement. After a study of all types of curves, it was found that the best results could be obtained by use of an equation having the general form:  $y = a \log (bx + 1) + c$ . Using this equation we were able to pass curves through the data of observation with gratifying closeness.

At the time this work was done, it did not seem necessary to stress the details of the mathematical processes. The calculated curves were needed only to insure accuracy; how they were obtained did not seem important. Nothing was said, therefore, about the manner in which the arithmetical values of the constants  $a$ ,  $b$ , and  $c$  of the given equation were derived. During the years intervening since this work was done, a number of inquiries have been received asking for information as to just how the values of the constants were obtained. In presenting the method briefly, we are deeply conscious of the fact that this discussion is purely mathematical, not physiological; the inquiries, however, come from plant physiologists. We hope it is not inappropriate, therefore, to supply the information through a journal which reaches those who seem most interested, and who may be most helped in connection with other studies of rates that change with time, if they happen to require the use of the same general equation.

Before taking up the problem of approximation in connection with the constants, it seems advisable to point out a few fundamental mathematical facts. If one selects *only three* data points through which to pass a curve, it is possible with an equation of three constants to pass any type of curve—circle, ellipse, parabola, or hyperbola—through the three points. In selecting the three data points out of a long series of observations, one may easily make a poor choice of one or more points. That is, the points selected for a trial curve may lie above or below the line of the final accurately fitted curve. If any point lies outside of this "best curve" it is a necessary and vital part of the analysis to discover such errors, and to correct for them by changing the constants which determine the position and path of the finally accepted curve. While a part of the process may seem like "trial and error," it is always possible to discover how much correction must be applied to which



constant to swing the curve into a new position, or to change its shape in some desired direction.

We feel that the simplest way to explain the procedure is by use of a concrete example taken from one of the earlier absorption studies, and to show just how the equation  $y = 29.6 \log (0.0078x + 1) + 4.38$  (see 3, p. 266, table II) came to have the values 29.6, 0.0078, and 4.38 for the constants  $a$ ,  $b$ , and  $c$ , respectively.

Given the equation:  $y = a \log (bx + 1) + c$ ,

To determine the value of the constants  $a$ ,  $b$ , and  $c$ , in a specific series of observed data to which this type of equation applies.

Since the method of approximation must be employed, the problem is first changed to a form involving only one of the constants.

To eliminate  $c$ :

$$y_1 = a \log (bx_1 + 1) + c \quad (1)$$

$$y_2 = a \log (bx_2 + 1) + c \quad (2)$$

Subtracting equation (1) from equation (2),

$$y_2 - y_1 = a \log (bx_2 + 1) - a \log (bx_1 + 1), \text{ or}$$

$$y_2 - y_1 = a \log \frac{bx_2 + 1}{bx_1 + 1} \quad (A)$$

In this manner constant  $c$  is eliminated from the equation.

The constant  $a$  may also be eliminated from the equation. In order to do this, it is necessary to have a second equation having only  $a$  and  $b$  as constants. An independent equation similar to (A) is readily obtained:

$$y_3 = a \log (bx_3 + 1) + c \quad (3)$$

Subtracting equation (2) from equation (3),

$$y_3 - y_2 = a \log \frac{bx_3 + 1}{bx_2 + 1} \quad (B)$$

Eliminate constant  $a$  by dividing (B) by (A):

$$\frac{y_3 - y_2}{y_2 - y_1} = \frac{\log \frac{bx_3 + 1}{bx_2 + 1}}{\log \frac{bx_2 + 1}{bx_1 + 1}} \quad (C)$$

In equation (C) the values of  $y_1$ ,  $y_2$ ,  $y_3$  are known ordinates corresponding to certain known values of  $x_1$ ,  $x_2$ ,  $x_3$ . We may now substitute these known values in equation (C) and carry out the operations to obtain the value of constant  $b$ , the only unknown quantity in the equation.

It is then easy, with  $b$  known, to substitute its value in equation (A) in which  $a$  becomes the only unknown quantity. With  $a$  known, the value of  $c$  may finally be found by substituting the determined values of  $a$  and  $b$  in equations (1), (2), or (3). To obtain most accurate results one should use six-place logarithms in making these computations.

The preceding steps present, in their simplest form, the mathematical steps involved in any case. The work may be carried to such a degree of refinement as to make the curve pass very precisely through any three points chosen for the calculations.

These values of the constants are to be regarded as approximations only. The reason for this is obvious; for in accumulating any given mass of data, there are many ways in which errors of observation may arise. These errors are inherent in the work, and are not entirely attributable to the investigator. Any value of  $y$  may be slightly above or below the true position of the curve that passes through the data with satisfactory precision. Some of the causes of irregular values of  $y$  have been considered in a previous paper (4). One is most likely to find, therefore, that the curve which passes through the three points chosen for the approximation may not pass through any other points in the entire series of observations, and not even satisfactorily close to them. In other words, this preliminary curve may afford a very poor "fit" to the data of observation. In the event that a very poor fit is obtained by the preliminary work on the constants, it becomes necessary to change the values of  $a$ ,  $b$ , or  $c$ , or of all of them, in such manner as to bring the curve into satisfactory agreement with the observations. The general problem is to swing the curve, or change its form just enough, to give it a location which represents a fair compromise with the irregular data, leaving some points above the curve, and others below the curve, with all points as close to it as possible.

In order to make such changes in the value of the constants intelligently, it will be well briefly to examine the effects of changing the values of each of the constants. The constant  $a$  determines in large measure the general upward rise of the curve from left to right. If it is found that the original curve from the three chosen points extends downward to the left *below* the observational data points, and rises *above* them to the right, the needed correction is obtained by *decreasing* the value of  $a$ . If the position of the curve is reversed with reference to the data as a whole, *i.e.*, too high at the left and too low at the right, the value of  $a$  must be *increased*. These changes tend to swing the curve into a position parallel with the data, although the curve may then lie *too low* or *too high*, even if it is parallel; for the change of  $a$  also displaces the curve *upward* or *downward* as it is increased or decreased.

After  $a$  has been adjusted to give a curve sensibly parallel with the line of data points, the whole curve is lifted or lowered into proper position by increasing or decreasing the value of  $c$ .

If, when the curve has been satisfactorily located at both left and right, the middle portion of the curve rises too high (above the compromise line of data points) the value of  $b$  must be decreased, and then new values of  $a$  and  $c$  must be determined. Or if the central region of the curve is too low, the value of  $b$  must be increased, and the necessary changes made in  $a$  and  $c$ . In

other words, changes in  $b$  change the radius of curvature, so that it is flatter, or more arched, as  $b$  decreases or increases.

Long experience with these problems leads us to suggest the following procedure as the best one to use in such work:

On accurately ruled coordinate paper, plot with the greatest possible accuracy all of the data points as determined by their abscissae and corresponding ordinates. Examine these along the entire line, to note any points that are obviously or apparently above or below a smooth curve through their midst. Choose values of  $y_1$ ,  $y_2$ , and  $y_3$  not suspected of being too high or too low—one of them near the left end, another near the right, and a third somewhere between, preferably nearer to the first than to the last point. Use these three values of  $y$ , with their abscissae values of  $x$ , in determining the value of  $b$  in equation (C). The value of  $b$  so determined is not likely to need change, but it may need changing, as in the following concrete example. The values of  $a$  and  $c$ , on the other hand, often have to be changed slightly to effect a satisfactory compromise with the data.

### Example

To illustrate the method, now, as a concrete problem in experimental work, we may take water absorption data obtained with seeds of Hickory King corn at a constantly maintained temperature of 35° C. The following data appear in a previous paper (3, p. 265, table I).

ABSORPTION PERIOD	WATER INTAKE (AIR DRY BASIS)
<i>min.</i>	%
15	5.76
30	7.10
45	8.34
60	9.31
90	11.09
120	12.85
180	15.67
240	17.95
300	19.87
420	23.21

Using time values as abscissae and percentage values as ordinates, the successive data points are plotted on accurately ruled coordinate paper. Examination of the plotted points shows that the 90-min. percentage is too low, and the 420-min. point too high with reference to the rest of the data. All other points lie on or near a smooth curve. We may now select three of these points for a tentative determination of the value of constant  $b$ , as follows:

$$x_1 = 15, y_1 = 5.76$$

$$x_2 = 120, y_2 = 12.85$$

$$x_3 = 300, y_3 = 19.87$$

Then the left side of equation (C) by substitution of these values, becomes:

$$\frac{y_3 - y_2}{y_2 - y_1} = \frac{19.87 - 12.85}{12.85 - 5.76} = 0.9901 +,$$

and the right side of equation (C),

$$\frac{\log \frac{bx_3 + 1}{bx_2 + 1}}{\log \frac{bx_2 + 1}{bx_1 + 1}} = \frac{\log \frac{300b + 1}{120b + 1}}{\log \frac{120b + 1}{15b + 1}}$$

If, now, we choose some value for  $b$ , as 0.007, and perform the mathematical operations of this last equation, the resulting value is 1.0237 -. This is greater than the true value, 0.9901 +, just obtained from the left side of equation (C). If we put  $b = 0.008$ , the resulting value is 0.9843 +, which is now less than the true value. Obviously the value of  $b$  lies between 0.007 and 0.008, and much nearer to the latter than to the former. If  $b$  is taken as 0.0078, the resulting value for the right side of equation (C) is 0.9916 +. This value is again slightly greater than the true value, consequently the value of the constant should be somewhat greater than 0.0078. If we put  $b = 0.00784$ , we now find that the value of the right side of equation (C) (last equation above), is 0.9901 -, a very close approximation to the true value of  $b$  obtained from the left side of equation (C).

Having obtained the constant  $b$ , substitute its value in equation (A) or (B), and the value of constant  $a$  is found to be 29.58 -. We can now substitute the values of both  $a$  and  $b$  in equation (1), (2), or (3) and determine the value of constant  $c$ , which is found to be 4.332. With these three values for the constants ( $a = 29.58$ ,  $b = 0.00784$ , and  $c = 4.332$ ) the curve now passes very precisely through the three chosen points ( $x_1, y_1$ ), ( $x_2, y_2$ ), and ( $x_3, y_3$ ).

Using the formula  $y = 29.58 \log (0.00784x + 1) + 4.332$  we may now compute the value of  $y$  for each value of  $x$  in the data given. These computed values are then set out in a horizontal line to represent the true curve. The three values used in the preceding computations agree exactly, of course, with the experimental data, since the curve was developed specifically for these three points. Above or below each computed  $y$  value of the curve, as it happens to lie, we write the corresponding observed value. In this 35° C. absorption series we find:

	7.10	8.34	9.31				15.67	17.95		23.21
Computed,	5.76	7.05	8.21	9.28	11.19	12.85	15.64	17.93	19.87	23.05
					11.09					

Most of the data of observation lie slightly above the curve, so it is obvious that the curve is somewhat too low, particularly at its left end. The choice

of 5.76 ( $x_1, y_1$ ) as one of the points in the curve has depressed that end of the curve, indicating that 5.76 per cent. is a low reading for the 15-minute value of absorption at 35° C. with the variety of corn used.

To raise the curve at the left end without changing the other chosen points (12.85 and 19.87) reduces the convexity of the curve as a whole, and requires a decrease in the value of  $b$ , which we said was not likely to need change. At a venture, the value of  $b$  is reduced to 0.0078. Now the new values of  $a$  and  $c$  must be determined by the methods already outlined, and a new set of  $y$  values computed for the entire series.

As the value 5.76 is being abandoned as a correct starting point for the curve, we substitute 0.0078 for  $b$  in equation (B)—not in (A)<sup>1</sup>—and find  $a=29.64$ . Now putting  $a=29.64$ , and  $b=0.0078$  in equation (2) or (3)—not in (1)<sup>1</sup>—we find  $c=4.346$ . Now proceeding to compute the new values of  $y$  for comparison with the observational data, we find:

	7.10	8.34	9.31			15.67	17.95		23.21	
Computed,	5.77	7.05	8.22	9.29	11.19	12.85	15.64	17.93	19.87	23.05
	5.76				11.09					

The convexity of the curve has been slightly reduced, but it still lies too low. One can see, also, that the calculated and observed values are closer together at the right than at the left. The general slope must be reduced by lifting the left end of the curve. This is accomplished by decreasing the value of  $a$  from 29.64 to 29.6. The corresponding value of  $c$ , derived now from equation (2), is 4.36. Again calculating the  $y$  values (with  $a=29.6$ ,  $b=0.0078$ ,  $c=4.36$ ) we obtain the following series:

		7.10	8.34	9.31			15.67	17.95	19.87	23.21
Computed,	5.78	7.06	8.23	9.30	11.20	12.85	15.64	17.92	19.86	23.04
	5.76				11.09					

The total effect of changing  $a$  has been to raise the left end of the curve by a small amount, and to depress the right end by a small amount, so as to make the curve now sensibly parallel to the observed data; but the curve is still too low, with seven values of the observations above, and only two below the computed values of the curve. The computed curve lies too low because it was *assumed* that the points 12.85 per cent. and 19.87 per cent. were without error of observation when the three original values were selected for the establishment of the curve. As it turns out, these values were too low by about 0.02. If we add this amount to the value of  $c$  (to lift the entire curve), we have  $c=4.38$ . A final computation of the  $y$  values now yields the following series:

	7.10	8.34				15.67	17.95		23.21	
Computed,	5.80	7.08	8.25	9.32	11.22	12.87	15.66	17.94	19.88	23.06
	5.76			9.31	11.09	12.85			19.87	

This distribution of the observations with reference to the computed

<sup>1</sup> Because  $y_1$ , which occurs in both (A) and (1), has been abandoned as too low.

values, five above and five below, is satisfactory, and nothing would be gained in any further attempts to adjust the constants. The curve accepted for this series of data, then, becomes  $y = 29.6 \log (0.0078x + 1) + 4.38$ .

The development of a considerable number of such curves involves much mathematical tedium unless one enjoys doing it. In accurate analyses it may be necessary, whether one enjoys it or not. We have tried to illustrate the derivation of the original constants, whereby approximate values may be obtained for the testing of fit. And we have shown how, by small changes first of  $b$ , then of  $a$ , and finally of  $c$ , the curve may be fitted, as accurately as the data permit, to the observed values. Such closely fitted curves are ideal for use in the computation of the rate of the process under investigation at any given moment within the limits of the curve.

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# ELECTRICAL RESPONSE OF *PHASEOLUS MULTIFLORUS* TO ELECTRICAL CURRENTS

W. S. REHM

(WITH FOUR FIGURES)

Several investigators have studied electrical polarization phenomena in multicellular plants. MARSH (1) found in the onion root and ROSENE (4) in branches of the Douglas fir that the effect of an applied current was dependent on the direction of the current flow. These investigators reported that applied potentials opposing the inherent potentials resulted in a temporary increase in the magnitude of the inherent potentials, while potentials applied in the same direction as the inherent ones produced a temporary decrease in magnitude. The present paper is concerned with a report of the effects of applied current on the inherent potentials of *Phaseolus multiflorus*. It was found as will be demonstrated in the following pages that, in general, the gross response to applied current in this particular plant is independent of the direction of current flow.

## Methods

The method for measuring the potentials and the conditions under which the experiments were performed were essentially the same as those described in previous papers (2, 3). Figure 1 illustrates the method of applying the current and measuring the potentials. In this diagram X and Y represent

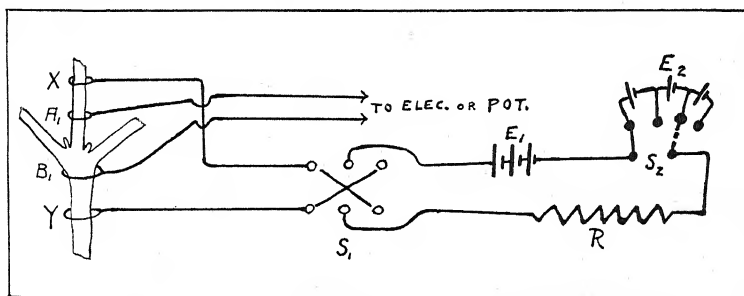


FIG. 1. Method of applying current. X and Y are contacts through which the current is sent. A<sub>1</sub> and B<sub>1</sub> are contacts between which the potential difference was measured. Elec. refers to electrometer and Pot. to potentiometer. For further explanation see text.

the contacts through which the current was applied and A<sub>1</sub> and B<sub>1</sub> represent contacts from which the inherent potentials were measured. E<sub>1</sub> represents a "B" battery of appropriate voltage and E<sub>2</sub> represents a series of 1.5-volt dry cells. By means of switch, S<sub>2</sub> the number of dry cells included in the circuit could be varied. R represents a large resistance (2 megohms). The



purpose of this large resistance was to prevent fluctuations in resistance of the plant from affecting appreciably the magnitude of the current flowing through the plant.  $S_1$  is a double-pole double-throw switch used for reversing the current.

It is to be noted that separate electrodes ( $\text{Zn}:\text{ZnSO}_4$ ) were used for measuring the potentials, which eliminates the possibility of errors from polarization effects at the electrodes.

In all the experiments reported in this paper the current was passed through the plant for 3-minute periods. Owing to the relatively long period of the instruments employed the first readings were not obtained until 30 seconds after turning off the current. For a more detailed analysis of these phenomena a different type of instrument must be used.

### Results

The following figures represent typical responses and in each type of experiment at least 5 plants were used. Figure 2A shows the effect of

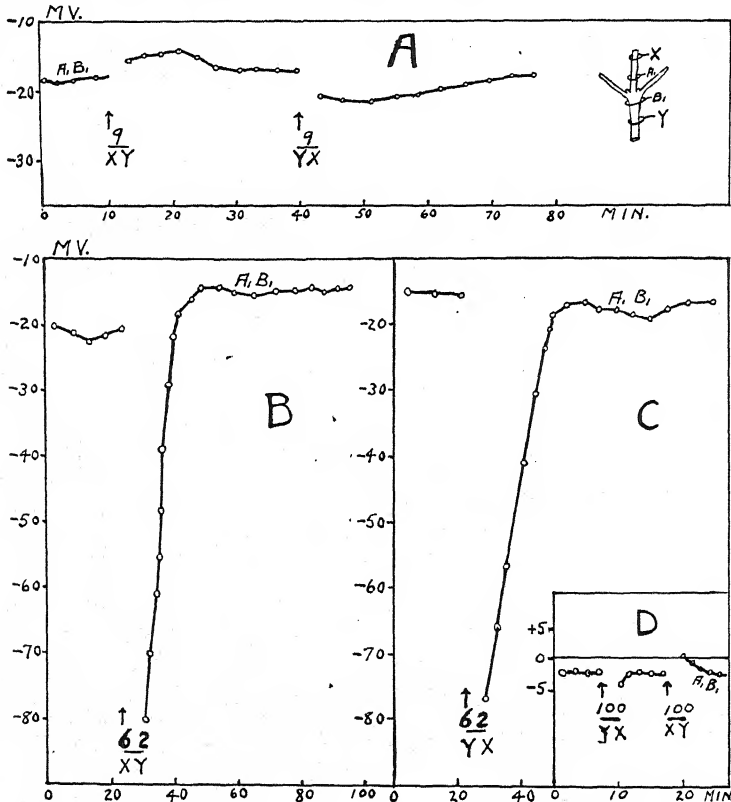


Fig. 2. Abscissa in millivolts. Ordinate, time in minutes. X was 2 cm. above  $A_1$ . Y was 2 cm. below  $B_1$ . The diagram in A showing the position of the contacts applies also to both B and C.

relatively small currents on the potential difference from  $A_1$  to  $B_1$  (referred to hereafter as  $A_1B_1$ ). At the time indicated by the first arrow, a current of 9 microamperes was sent from X to Y; and at the time indicated by the second arrow, a current of the same magnitude was sent in the opposite direction from Y to X. The current from X to Y caused a temporary decrease in the absolute magnitude of the potential  $A_1B_1$  while sending the current from Y to X resulted in a temporary increase in the magnitude of the potential.

On the other hand, currents well above the threshold value produced a marked increase in the magnitude of the potentials irrespective of the direction of the current.

In figure 2 B and C, currents of 62 microamperes were sent from X to Y and from Y to X. In both plants there was a large increase in the magnitude of the potential  $A_1B_1$ . After sending the current from Y to X, opposing the inherent potential, the potential  $A_1B_1$  was maintained at a slightly greater magnitude than before the application of current, whereas after the current passed from X to Y the potential  $A_1B_1$  was maintained at a lower magnitude (*i.e.*, nearer the base line). The potentials returned very gradually to their original magnitude. In fact this return was so slow that spontaneous variations of the potential prevented any very accurate determination of the time interval involved.

That this effect is dependent on the living cells is demonstrated by figure 3D which shows the effect of a current of 100 microamperes on the node potential  $A_1B_1$  of a plant killed by immersing it over night in a formalin solution. In this case the effects were of small magnitude and depended on the direction of the current. When the current opposed the inherent potential, the magnitude was slightly increased; and when it was applied in the same direction as the inherent potential the latter was decreased.

The typical response of the petiole potentials to an applied current is shown in figure 3. This figure shows the effect of currents of 2, 6, and 30 microamperes on the potentials  $O_1P_1$  and  $O_1'P_1'$ . In each case the current

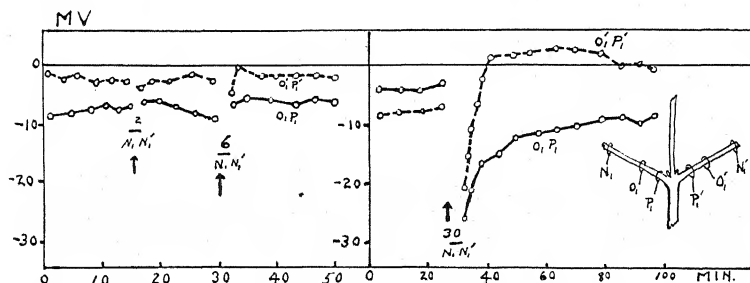


FIG. 3.  $N_1$  and  $N_1'$  were placed at the apex of the petioles,  $O_1$  and  $O_1'$  were placed 1.5 cm. above  $P_1$  and  $P_1'$ .

passed downward from  $N_1$  to  $P_1$  in the one petiole, and upwards from  $P_1'$  to  $N_1'$  in the other petiole. If the response to the applied current should depend on the direction of the current, then the response of  $O_1P_1$  would be different from that of  $O_1'P_1'$ . Two microamperes produced practically no observable effect while 6 microamperes produced an observable effect dependent on the direction of the current. However with 30 microamperes the primary effect was independent of the direction of the current. A large increase was produced in the magnitude of both potentials. The potential  $O_1'P_1'$ , after returning to a relatively constant level, was less negative than before, whereas  $O_1P_1$  was more negative than before.

Figure 4A shows the effect on the 3 node potentials [ $A_1B_1$ ,  $A_1P_1$ , and  $A_1'P_1'$ , see previous paper (2)] of sending the current from  $O_1'$  to X. In

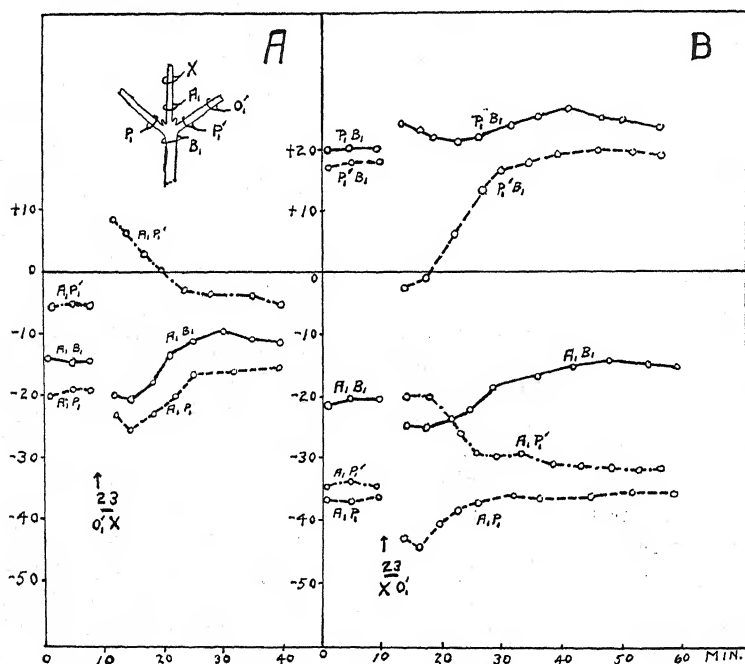


FIG. 4. X was placed 2 cm. above  $A_1$ , and  $O_1'$  was 2 cm. above  $P_1'$ . The diagram in A also applies to B.

this experiment  $A_1B_1$  became more negative (an increase in absolute magnitude) as might be predicted from the results presented in figure 1 since the current flowed through most of the region from  $A_1$  to  $B_1$ .  $A_1P_1$  shows a change similar to  $A_1B_1$  which again would be expected since the region through which the current flowed is roughly common to both  $A_1P_1$  and  $A_1B_1$ . The response of the potential  $A_1P_1'$ , although it decreased in magnitude, is not actually an exception to the general rule, as the following

analysis demonstrates. The response of  $A_1P_1'$  is made up of two components, namely  $A_1$  to the node and  $P_1'$  to the node. Since both of these responses should result in an increased basal positivity they would be oriented in opposite directions and the effect on  $A_1P_1'$  would depend upon the difference of the two effects. Figure 4B shows the response of the node potentials plus those of  $P_1'B_1$  and  $P_1B_1$ . Since the response of  $P_1'B_1$  is greater than that of  $A_1B_1$  the direction of the response of  $A_1P_1'$  ought to be determined by  $P_1'B_1$  which is actually the case. An inspection of the figure shows that the effect on  $A_1P_1'$  is a quantitative summation of the effects on  $P_1'B_1$  and on  $A_1B_1$ .

In this figure  $P_1B_1$  shows an increased apical positivity which was a typical response of this potential to current sent in either direction through the main stem and opposite petiole.

### Discussion and conclusions

The results presented in this paper demonstrate that in *Phaseolus multiflorus* relatively small currents produce an effect that is dependent on the direction of the current, while currents of greater magnitude produce in the main axis and petioles an increase in basal positivity. In a former paper (3) it was demonstrated that decapitation produced a similar increase in basal positivity. Both results demonstrate a latent oriented mechanism, inherent in the plant, capable of producing comparatively large electrical potentials. This is roughly comparable to the situation in nerve and muscle. In these tissues stimuli produce an electrical response that is determined by an inherent oriented mechanism. The analogy suggests that this electrical response may play a part in the economy of this plant.

The writer has demonstrated in previous work (2, 3) that in *Phaseolus multiflorus* there is an intimate relation between the orientation of bioelectric potentials and morphology. When the plant changes from one morphological state to another there is a concomitant reorientation of electrical potential patterns. Interestingly in this connection, the main electrical polarity of *Phaseolus multiflorus* was one of basal positivity.

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## TRANSPORT OF INORGANIC IONS IN POLAR PLANT TISSUES

F. W. WENT

In a short note some remarkable parallelisms between the polar auxin transport in stems and salt accumulation by many plant cells were pointed out (8). It was suggested that both phenomena were an expression of the same basic property of active cells, namely, their adenoid activity. In this connection the question arose whether inorganic salts were transported inside plant organs by the same mechanism which causes the auxin to move from apex to base only. Some phenomena seemed to support this idea of polar movement of bases and acids in general. The excretion from roots is acid whereas the exudate from leaves is basic.

To get data on the polar movement of inorganic ions inside stems it was deemed necessary to apply and test for exceedingly small amounts; for experiments of WENT and WHITE (9) had indicated that the polar transport mechanism moves very small amounts of auxin only: in *Avena* coleoptiles not more than  $10^{-5}$  mg. of indoleacetic acid is transported from apex to base per  $\text{mm.}^2$  per hour. Thus quantities of the same order of magnitude should be determinable of each ion to be tested. Since it is possible to induce such strong radio-activity in certain elements commonly found in plants, that amounts of less than  $10^{-5}$  mg. can be measured with a Geiger counter, it was decided to try the transport of some radioactivated elements in polar plant tissues. So far these experiments had not been carried out under exclusion of the transpiration stream (3). Through the courtesy of the divisions of Physics and Plant Nutrition of the University of California, and especially through the kind help of Mr. P. R. STOUT and Dr. F. SKOOG, the writer was enabled to carry out some experiments in Berkeley.

Different samples of radioactive bromine as  $\text{Br}^-$ , radioactive phosphorus as  $\text{PO}_4^-$  and radioactive sodium as  $\text{Na}^+$  were tested on *Avena* coleoptiles and *Helianthus* hypocotyls and epicotyls. All transport experiments were carried out in a physiological dark room kept at  $25^\circ \text{C}$ . and 85 per cent. humidity, in which also the auxin determinations were made. The radio-activity was determined in an adjoining room with a Geiger counter in which the samples could be brought in very close proximity to the electrodes. The samples were wrapped in cellophane to prevent contamination of the counter surface. Of each sample, readings were taken at 1-minute intervals for periods of at least 5 minutes to get an idea of the significance of the values measured. This was especially necessary when the counts ran close to the "background."

Two types of experiments were carried out. The first and simpler one was less satisfactory. Either to the apical or basal cut surface of a cole-

optile or hypocotyl a block of agar soaked in a very dilute solution of the radioactive material was applied. After 3 to 6 hours the stems were cut into segments of 3 or 5 mm. length, and the radioactivity of each section was determined. This procedure had several disadvantages. In the first place the possible accumulation of the ions by the cells made a clear interpretation of the results rather difficult. And in the second place it was not known whether the auxin transport was polar at the time of ion transport, although in a control experiment done with the same material in the same darkroom the transport of indoleacetic acid was perfectly polar. The results of this type of experiment did not show any consistent preferential direction in the transport of  $\text{Br}^-$  and  $\text{Na}^+$  in *Avena* coleoptiles and *Helianthus* stems.

The most significant results were obtained with the following technique. Agar blocks of standard size ( $2.5 \times 2.5 \times 1.5$  mm.) were soaked in the radioactive solutions. Then 5-mm. sections of coleoptile or hypocotyl were placed on them, either with their base or with their top down (it has been shown by various authors, *e.g.* (5), that gravity does not influence the polar longitudinal auxin transport). On each section was then placed another standard agar block soaked in a solution of 5 mg. indoleacetic acid per liter of water. After 3 to 6 hours the lower blocks were tested for auxin and the upper blocks for radioactivity. In this way the polarity of auxin transport was determined in the same sections simultaneously with the inorganic ion movement. In every one of the 120 sections tested for auxin the polarity was absolute: no trace of indoleacetic acid was found when it had been applied from the base, whereas strong curvatures were obtained when the basal block was analyzed after application of auxin from the apical cut surface of the section. Thus the traces of radioactive material had not influenced the physiological behavior of the tissues.

In table I the results of these experiments are summarized. The figures give the number of counts per minute, from which the background has been subtracted. Therefore, below about 1000, the concentration of the radioactive ion is directly proportional to the figure given. Any figure above 4 can be taken to indicate the presence of significant amounts of radioactive material. At higher values the standard deviation amounts to about 4 per cent.

Although it makes little difference for the indoleacetic acid transport, whether the tissues are cut nearer the apex or the base of the coleoptile or hypocotyl, in the inorganic ion transport we observe a consistent and significantly larger amount moving through basal than through apical parts of the same organ. The differences are especially marked for the  $\text{PO}_4^-$  movement. Care was always taken, therefore, to compare sections from corresponding regions of the plant, and the transport through more apical and





more basal regions was determined separately. But no clear evidence of polarity in the inorganic ion translocation could be detected. Only in the case of the most apical hypocotyl sections some indication of polarity was found, but this is not sufficient to account for polarity in general. As mentioned before, *all* parts of the coleoptile and hypocotyl have an equally pronounced polarity in their indoleacetic acid transport, so that in *all* sections a polar inorganic ion transport had to be expected if transport of the two types of substances were caused by the same mechanism. Although quantitatively the experiments leave many questions to be answered, one conclusion seems well established. *In the same tissues simultaneously there occurs a polar auxin transport and a non-polar movement of inorganic ions.* Thus there is no escape from the fact that the movement of auxin and of  $\text{Na}^+$ ,  $\text{Br}^-$  and  $\text{PO}_4^-$  is controlled by completely different mechanisms. On different grounds CLARK (2) came to the same conclusion, since sodium glycocholate completely inhibited polar auxin transport while it had no influence on the accumulation of  $\text{Br}^-$  by barley roots.

One may envisage this as a fundamental difference in the locomotor mechanism for the movement of these substances, or simply as a difference in the path of transport. If (1) we accept VAN DER WEIJ's notion (6) of auxin movement in the surface layer between protoplasm and cell wall, where an adsorbed film of auxin would move in the manner exemplified by VAN DEN HONERT's model (4) and if (2) the polarity of the auxin transport were located somewhere along this surface layer, then only substances which positively adsorb on this boundary layer could be transported in a polar way. Since auxin, indoleacetic acid and similar growth-promoting substances are lipid soluble and will positively adsorb on a water-air interface, whereas the ions tested lack both these properties, the latter could not move according to a VAN DER WEIJ-VAN DEN HONERT scheme.

Whatever explanation one accepts for the discrepancies between the mode of transport of auxins and inorganic ions, it is clear that the accumulation mechanism for inorganic ions and the polar transport mechanism for auxin are not identical, and must be located in different parts of the cell. Another discrepancy between these two mechanisms may be pointed out. The cell is able to accumulate both anions and cations. The rather meager experimental evidence (1, 7) however, indicates that in the polar transport mechanism all acids which were tested moved from apex to base and all bases from base to apex. Further work, including many more substances, which must be measurable in minute quantities, will have to confirm this conclusion.

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## THE ESSENTIALITY OF CERTAIN ELEMENTS IN MINUTE QUANTITY FOR PLANTS WITH SPECIAL REFERENCE TO COPPER

D. I. ARNON AND P. R. STOUT

The recent discoveries of the importance of small amounts of boron, manganese, copper, and zinc (1, 2, 4, 7, 13) in the physiology of higher plants, and in a number of soil-plant problems of considerable agricultural importance, have added interest to studies on the rôle of these elements in plant nutrition. The most obvious and important question is whether or not these elements are indispensable to the growth of plants.

Recent investigations in this laboratory (12) on the importance of certain metals in minute quantity in the economy of higher plants, have resulted in the development of an experimental technique which makes possible a consistent and reproducible demonstration of the essentiality of copper, manganese, and zinc for the growth of higher plants. Although the details of the procedures will be described elsewhere (12) it is desired to present at this time some considerations and conclusions which may be of general interest.

It was deemed desirable to test experimentally the essentiality of each element according to the following criteria: an element is not considered essential unless (a) a deficiency of it makes it impossible for the plant to complete the vegetative or reproductive stage of its life cycle; (b) such deficiency is specific to the element in question, and can be prevented or corrected only by supplying this element; and (c) the element is directly involved in the nutrition of the plant quite apart from its possible effects in correcting some unfavorable microbiological or chemical condition of the soil or other culture medium. From that standpoint a favorable response from adding a given element to the culture medium does not constitute conclusive evidence of its indispensability in plant nutrition.

Conclusive proof of the indispensability of the so-called microelements, that is, elements required in minute amounts by plants, can be obtained by the use of artificial culture media (the water culture technique was used in this investigation), provided special procedures are employed to remove incidental impurities. The physiological importance of these contaminants is illustrated by the finding that young tomato plants grown in Pyrex glass containers with nutrient solutions deficient in zinc gave a measurable response to the addition of 1 gamma (0.001 mg.) of zinc to a plant (this gave a zinc concentration in the culture solution of 5 parts per billion). This response to a minute amount of zinc as well as similar responses to copper and manganese were consistently obtained only when redistilled water and purified chemicals were used. The metal content of ordinary distilled water was reduced by redistillation, using

a Pyrex glass condenser, from 10 to 100 to consistently less than 0.2 part per billion. The metal impurity derived from salts was reduced, by using a modification of STEINBERG's procedure (11, 12) from 15 to 150 to consistently less than 0.1 part per billion.

The experimental demonstration of the essentiality of microelements for plant life is frequently rendered difficult not only because plant needs may be satisfied by incidental contaminants, but also because different species vary greatly in their requirements. In this laboratory, for example, it was repeatedly found that a standard culture solution to which no manganese or boron was added produced excellent barley plants but gave severe boron and manganese deficiency symptoms in tomato plants. It may be advantageous therefore to select for experimental purposes plants having relatively high requirements for the microelement studied. For any one species the onset of deficiency symptoms may be hastened by reducing the reserve in the seed, either by using seed obtained from plants grown with a limited supply of microelements (5) or by early removal of the seed from the seedling (10). Thus the extent to which it is necessary to purify the culture medium in order to produce deficiency symptoms may be reduced through a suitable selection and preparation of experimental plants.

Since it cannot be assumed that any purification procedure has completely removed a given element from the nutrient medium, the conclusions from a negative result should best not be drawn with finality. When an element is omitted from a carefully prepared and purified artificial culture medium without seemingly hindering the growth and reproduction of plants, it need not be concluded that it plays no essential function in the physiology of plants. Regardless of the care exercised in the preparation of the culture medium, a minute, but physiologically significant, impurity may have persisted and needs to be considered as well as the reserves contained in the seed. It would seem best in such cases to determine analytically, whenever possible, the upper limit of impurity that may be present in the culture medium, and express the results of the experiment on a quantitative basis; that is, that with a certain upper limit of supply of a given element, no response is obtained with certain species of plants. It is possible that the attainment of a greater degree of purification of the culture medium by means of improved experimental procedures, the use of seeds having a low content of, or the selection of plants having a high requirement for, a particular element may give different results.

If these views are accepted, there can be no *a priori* objection to regarding almost every element in the periodic table, and particularly those most frequently encountered in plant products, as susceptible of being shown at some time to be essential for plants. (This is in accord with similar views expressed by HOPKINS (3).) What can be stated definitely at the present time

is that a given element is essential or not, depending on whether the plant requires it in an amount greater than that present in the culture medium as a result of contamination. It was found, for example, that if the combined zinc, copper, lead, cadmium, and mercury content in the nutrient solutions was reduced to less than 0.1 of a gamma (0.0001 mg.) to a plant, as determined by analyzing the culture solution (12), very severe deficiency symptoms were obtained. In this case a definite response was obtained from supplying 2 gammas of copper and 2 gammas of zinc, but no further improvement was produced by supplying to a plant 0.5 of a gamma each of lead, cadmium, and mercury. These results, while showing the indispensability of zinc and copper for the growth of tomatoes in amounts larger than those left behind in the purified culture medium, were interpreted as permitting no final conclusion as to the rôle of cadmium, lead, and mercury in the nutrition of tomatoes. The possibility that these elements may be required in amounts smaller than the incidental impurities which could not be removed from the culture solution by the present technique, cannot be *a priori* excluded.

This quantitative approach to the problem of essentiality of microelements is regarded not as a mere theoretical generalization, but as a point of view conducive to a search for more refined analytical methods and procedures for growing plants, which would make it possible to investigate the rôle of a number of new elements in plant nutrition.

After experimentally producing the characteristic deficiency symptoms for an element, the next step was to show that it had a specific and direct effect on the physiology of the plant. The question of the essentiality of copper was a case in point. SOMMER, (9) and LIPMAN and MACKINNEY (6) have independently demonstrated the necessity of copper for the vegetative growth and reproduction of higher plants. It was recently suggested, however, that the observed response to copper may have been due to an indirect influence, *i.e.*, its antagonistic or balancing effect on other elements (8). Attention was also called to the desirability of confirming the specificity of copper and excluding the possibility that some other element capable of existing in different valence states, could replace copper (2).

The specific and direct effect of copper on the growth of tomatoes was demonstrated as follows: Typical copper deficiency symptoms in tomatoes (very much stunted growth of shoots and exceedingly poor root development, dark bluish-green color of foliage, curling of leaves, and absence of flower formation) were produced by growing the plants in culture solution in two-liter Pyrex beakers, using purified salts and redistilled water (12). (Copper deficiency symptoms could be produced only if water redistilled over a Pyrex condenser was used. Ordinary distilled water contained enough copper to satisfy the needs of tomatoes, even when purified salts were used.) The de-

velopment of the deficiency symptoms was prevented by supplying a plant with 2 gammas of copper as  $\text{CuSO}_4$ .

To test the possible antagonistic effect of copper on other elements, as distinguished from its direct effect on plant growth (9), plants were grown in solutions supplied in one case with only three microelements: boron, manganese, and zinc, and in another case with these plus 20 additional ones, including lead, mercury, arsenic, and selenium. It was found that the onset, type, and severity of copper deficiency symptoms were the same in both cases. The additional group of 20 elements included elements capable of existing in different valence states, such as molybdenum, vanadium, chromium, nickel, cobalt, titanium, and tungsten, none of which were found capable of replacing copper.

Leaves of young tomato plants showing copper deficiency symptoms were sprayed with a very dilute  $\text{CuSO}_4$  solution (0.02 parts per million of copper). This brought about recovery and the resumption of normal growth of both shoots and roots. These findings, showing the direct effect of copper on the plant as distinguished from its possible effect on the root environment, were interpreted as providing a final link in the chain of evidence of the essentiality of copper for the growth of tomato plants.

The small quantitative requirement of copper and other elements for plant life need not detract from an appraisal of their importance in the physiology of higher plants. There is no choice between essential elements. In that sense the fraction of a milligram of copper is just as indispensable as several hundred milligrams of a "major" element like potassium, since in the absence of either of them a tomato plant will fail to complete its life cycle. It is for this reason that the term "minor elements" frequently used to designate the group of elements essential for plants in minute quantity, should not lead to the inference that these elements occupy a position of secondary importance in the nutrition of higher plants.

The fact that most plants are injured by relatively very low concentration of microelements (for example injury to tomatoes was obtained from 2.0 parts per million of copper in the culture solution) adds interest to the search for the explanation of their function in the plant.

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# ALKALOIDS FROM *SANGUINARIA CANADENSIS* AND THEIR INFLUENCE ON GROWTH OF *PHYMATOTRICHUM* *OMNIVORUM*

GLENN A. GREATHOUSE

(WITH ONE FIGURE)

## Introduction

*Sanguinaria canadensis* L. is reported by TAUBENHAUS and EZEKIEL (6) to be highly resistant to the *Phymatotrichum* root rot. It has long been of interest on account of its alkaloidal content. DANA (1) isolated the alkaloid, sanguinarine, from this species in 1827. Since that time chelerythrine, protopine, and  $\beta$ - and  $\gamma$ -homochelidonine have been isolated from the rhizome and roots (5). For these reasons this plant seems an exceptionally good object for investigation in connection with the theory of alkaloids as a possible factor in the resistance of some plants to *Phymatotrichum omnivorum* (Shear) Duggar (3, 4). In the work reported here certain alkaloids were isolated from crude extracts of roots and rhizomes of *S. canadensis* and incorporated in various concentrations into the substrates ordinarily used for the pure culture of *P. omnivorum*. The effect of these substances on the growth and development of the fungus are discussed.

## Materials and methods

Powdered rhizome and root tissue of *Sanguinaria canadensis* was purchased from Eimer and Amend Co. Two kilograms of this ground tissue was thoroughly extracted with acidified (acetic acid) alcohol, following the method of FISCHER (2) for the separation of the alkaloids. The procedure of extraction, separation, etc., is shown diagrammatically in figure 1. The suggestions of the original paper were followed in separating protopine and chelerythrine from sanguinarine.

## Results

Three alkaloids were separated from fractions BC and BS (figure 1). FISCHER found  $\beta$ - and  $\gamma$ -homochelidonine in fraction BSC, but in this study nothing of a crystalline form was secured; however, this fraction did yield a small amount of syrup of alkaloidal nature.

Alkaloids with the following physical properties and chemical composition were separated and studied for their effects on the growth of *P. omnivorum*. Alkaloid no. 1 (fraction BC and BS) crystallized from alcohol in colorless groups of needles, melting point  $212^{\circ}$  C.<sup>1</sup>; dissolved in alcohol, ether, chloro-

<sup>1</sup> All melting points are corrected.

form, forming solutions that were fluorescent. The hydrochloride was deep red in color. Chemical composition: Found,<sup>2</sup> C, 68.48 per cent.; H, 4.30 per cent.; N, 3.94 per cent. Calculated for sanguinarine ( $C_{20}H_{15}O_4N \cdot H_2O$ ), C, 68.34 per cent.; H, 4.31 per cent.; N, 3.99 per cent. The concentration found for this alkaloid was approximately 0.2 per cent.

Alkaloid no. 2 (fraction BS) crystallized from alcohol in colorless prismatic leaflets, melting point  $206^\circ C$ .; readily soluble in chloroform, sparingly soluble in alcohol and ether. The hydrochloride formed by the addition of

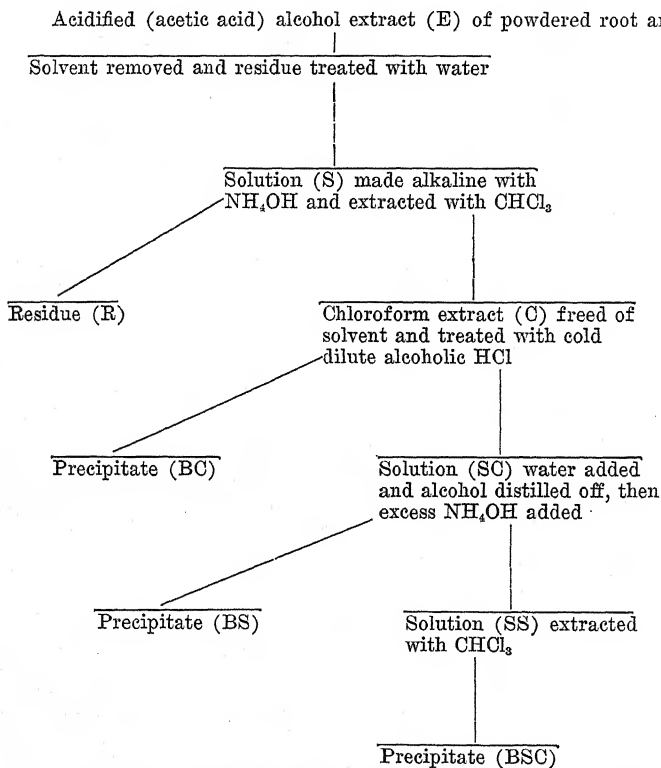


FIG. 1. Method of FISCHER for the separation of the alkaloids of *Sanguinaria canadensis*.

hydrochloric acid was citron-yellow in color, with needle-shaped crystals. Chemical composition: Found, C, 63.22 per cent.; H, 5.05 per cent.; N, 3.63 per cent. Calculated for chelerythrine hydrochloride ( $C_{21}H_{17}O_4N \cdot HCl \cdot H_2O$ ), C, 62.73 per cent.; H, 5.02 per cent.; N, 3.49 per cent. The concentration of this alkaloid was 0.1 to 0.2 per cent.

<sup>2</sup> Average of duplicate determinations made by Arlington Laboratories, Arlington, Virginia.

Alkaloid no. 3 (fraction BC) crystallized from chloroform by addition of a small amount of alcohol, formed monoclinic crystals, melting point  $208^{\circ}\text{C}$ .; very slightly soluble in alcohol, ether, benzene, and ammonium hydroxide; and soluble in acetone. Chemical composition: Found, C, 67.85 per cent.; H, 5.45 per cent.; N, 4.09 per cent. Calculated for protopine ( $\text{C}_{20}\text{H}_{19}\text{O}_5\text{N}$ ), C, 67.96 per cent.; H, 5.42 per cent.; N, 3.97 per cent. The concentration of this alkaloid was 0.01 to 0.05 per cent.

The effect of these alkaloids on the growth of *P. omnivorum* was tested in the manner reported by GREATHOUSE and WATKINS (4). The results are presented in table I.

### Discussion

Alkaloids having the physical properties and chemical composition of sanguinarine, chelerythrine, and protopine were isolated from *Sanguinaria canadensis*. All of these alkaloids, with the exception of protopine, were found to be present in the rhizome and root tissues in far greater concentration than that which prevents growth of *P. omnivorum*. The concentration of protopine found in the tissue was great enough to decrease fungous growth from 369 mg. to 81 mg. Sanguinarine completely prevented growth of the organism at a concentration of 2.5 p.p.m. Chelerythrine was not so toxic, permitting a growth of the fungus in three weeks of 3.4 mg. at a concentration of 10 p.p.m. Protopine under similar conditions yielded 81 mg. of

TABLE I  
INFLUENCE OF ALKALOIDS ISOLATED FROM *Sanguinaria canadensis* ON  
GROWTH OF *Phymatotrichum omnivorum*

COMPOUND	DRY WEIGHT (MG.) OF FUNGUS IN NUTRIENT SOLUTION + FOLLOWING CONCENTRATIONS OF ALKALOID			
	100 P.P.M.	50 P.P.M.	10 P.P.M.	2.5 P.P.M.
	mg.	mg.	mg.	mg.
Sanguinarine .....	0	0	0	0
Chelerythrine .....	0	0	3.4	73
Protopine .....	81	137	308	356
Control = 369 mg.				

fungous growth at 100 p.p.m. This study clearly shows that alkaloids, even from the same plant, are not equally inhibitive to the growth of *P. omnivorum*.

Experimental evidence suggests strongly that the alkaloids in roots and rhizomes of *Sanguinaria canadensis* constitute an important factor in the resistance of this plant to *Phymatotrichum* root rot.

### Summary

Sanguinarine, chelerythrine, and protopine have been isolated from roots and rhizomes of *Sanguinaria canadensis* by the method of FISCHER. Sangui-

narine has been shown experimentally to prevent the growth of *Phymatotrichum omnivorum* at a concentration as low as 2.5 p.p.m. Chelerythrine and protopine were less toxic than sanguinarine. There is considerable indication from this work that alkaloids play an important rôle in the resistance of *Sanguinaria canadensis* to *Phymatotrichum* root rot.

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## SEASONAL SUGAR VARIATIONS IN ALFALFA

J. C. IRELAND

(WITH ONE FIGURE)

Ten years after planting, it is assumed that alfalfa plants have sufficient hardiness to be considered more than ordinarily vigorous, especially in the extremes of Oklahoma seasons. MEGEE (2) doubts that winter hardiness may be caused by physiochemical differences in alfalfas. He concludes that electrical conductivity indicates the degree of hardiness of alfalfa plants and that winter hardiness is an hereditary factor. On the other hand, GREAT-HOUSE and STUART (1), studying clovers, show that adaptability to cold is indicated by the unfreezable-freezable water ratio, osmotic pressure value, and the greater concentration of total sugars is in the hardier selections. Selecting seven row-plantings of ten-year-old alfalfa plants, the heritable phases of hardiness may be considered about equal. The object of this investigation has been to determine whether there is a seasonal variation in the sugar concentration and just how much environmental factors enter into the length of life of plants.

Weekly determinations of sugar have been made since October, 1937. A Zeiss hand sugar refractometer was used to make the readings. Sap was expressed from the crown of the plant by means of a heavy pair of pliers. A few drops of sap provide enough for a reading, and there is no deterioration or delay. The percentage reading on the scale of the refractometer is comparable to the total sugar concentration. Sampling was completed each Monday morning as a field routine. Soil temperatures were recorded with a thermograph. The bulb was placed vertically, and the record indicates the average for the top twelve inches of soil. Weekly averages were determined by means of a planimeter.

One hundred and forty alfalfa plants were transplanted to each row in the spring of 1928. The surviving plants have been used for this determination. Saskatoon came from the Experiment Station in Canada, and it was probably a selection among several by Dr. L. E. KIRK from his Asiatic collection. Forty-four of the original 140 had survived. Oklahoma Common was from a well-known seed grower in northern Oklahoma. Utah was from an original forty-year strain of that state, 57 of the original 140 surviving. Dakota, Cossack, and Grimm alfalfas came from North Dakota.

In order to determine the relative differences in young and old alfalfa plants, samples were taken from five reputedly hardy alfalfas. These are recorded in table II, and the determinations were made during the last two weeks in May. The names of these selections indicate the source of the seed, which was planted in February, 1938.

TABLE I  
CONCENTRATIONS OF SOLUBLE SOLIDS IN ALFALFA CROWN SAP

DATE	SASKATOON 1122	OKLAHOMA COMMON	UTAH 2561	DAKOTA 552	COSSACK U.S.D.A.	COSSACK 2441	GRINN U.S.D.A.	AVE. PER- CENTAGE
	%	%	%	%	%	%	%	%
Oct. 4	9.0	15.0	8.0	9.5	8.5	16.0	14.5	11.5
Oct. 11	12.0	8.0	6.0	6.0	5.0	12.0	10.0	8.4
Oct. 18	11.0	10.0	13.0	9.3	6.0	15.0	11.0	11.0
Oct. 25	11.0	11.0	17.0	14.0	11.5	13.0	15.0	13.2
Nov. 1	18.0	16.5	17.0	10.0	12.0	14.5	14.0	14.5
Nov. 7	17.0	13.6	20.0	18.0	14.6	17.6	14.0	15.5
Nov. 15	17.2	10.0	16.8	13.0	12.5	15.0	20.0	15.0
Nov. 22	12.5	16.0	10.5	11.0	14.0	16.5	20.5	14.4
Nov. 29	20.5	13.0	13.5	18.0	15.4	19.0	18.5	16.8
Dec. 6	23.0	6.0	12.4	13.5	11.0	11.5	18.0	12.2
Dec. 16	20.5	22.0	16.5	19.5	14.0	18.0	14.5	18.2
Jan. 4	20.5	13.5	14.0	17.5	13.0	13.5	21.0	16.1
Jan. 10	18.5	15.0	16.0	11.0	10.5	16.5	9.5	13.8
Jan. 17	11.5	7.3	8.5	12.0	7.0	9.6	7.5	8.6
Jan. 24	19.0	14.2	12.0	18.5	13.2	11.8	18.2	15.3
Jan. 31	11.4	14.5	18.0	11.8	15.5	17.0	16.6	14.9
Feb. 7	21.4	14.5	22.4	13.5	10.8	18.0	11.1	15.2
Feb. 14	9.2	12.0	13.8	13.0	13.2	10.6	12.1	12.8
Feb. 21	12.0	14.5	11.6	9.4	9.0	10.6	8.7	10.8
Feb. 28	13.2	8.4	13.0	10.4	13.6	8.0	12.2	11.4
Mar. 7	8.4	10.2	11.4	7.5	7.0	11.2	13.4	9.9
Mar. 14	8.6	8.6	8.2	6.4	7.0	13.2	8.2	8.6
Mar. 21	7.7	6.0	9.5	8.6	8.4	9.0	5.5	7.8
Mar. 28	9.4	6.2	7.0	7.6	10.0	7.8	12.8	8.7
Apr. 4	13.0	11.4	10.4	13.2	10.6	7.4	10.6	10.9
Apr. 11	12.4	11.6	14.2	8.8	13.0	10.2	12.8	11.8
Apr. 18	12.8	10.4	10.2	13.0	10.4	12.4	11.8	11.5
Apr. 25	8.0	7.8	6.4	9.8	6.8	5.4	7.6	7.4
May 2	5.0	5.8	8.4	6.4	9.8	6.8	12.0	8.0
May 9	18.5	11.2	14.4	11.8	9.8	6.0	14.2	12.2
May 16	7.6	6.2	12.0	9.4	7.4	9.6	13.0	9.3
May 23	4.6	3.8	8.1	10.2	8.4	5.3	4.5	6.4
May 30	9.4	8.6	9.0	8.8	10.8	9.2	7.6	9.1
Average percentage	12.9 ± .55	11.0 ± .51	12.4 ± .49	11.5 ± .43	10.4 ± .41	12.0 ± .47	12.8 ± .48	11.8
Standard deviation	4.88 ± .39	4.36 ± .36	3.98 ± .33	3.65 ± .30	3.47 ± .29	4.02 ± .33	4.08 ± .34	
Surviving hills from 140 in 10 years	44	35	57	50	38	47	48	

## Results

Table I summarizes the readings of sugar concentrations in percentages of the total soluble solids.

The uniformity of sap concentration throughout the winter seems to be the most important factor in the length of life. Oklahoma Common is an example of a selection having comparatively low resistance. During a warm week, the first of December, the reading was 6 per cent. This was followed by one of the coldest weeks of the winter, and the reading was 22 per cent. During the last half of March, temperatures were unusually high, and the reading was about 6 per cent. An April freeze and snow storm brought the soil temperatures almost to freezing, and the cell sap concentration almost doubled. A glance at other selections for these periods indicates a less widely

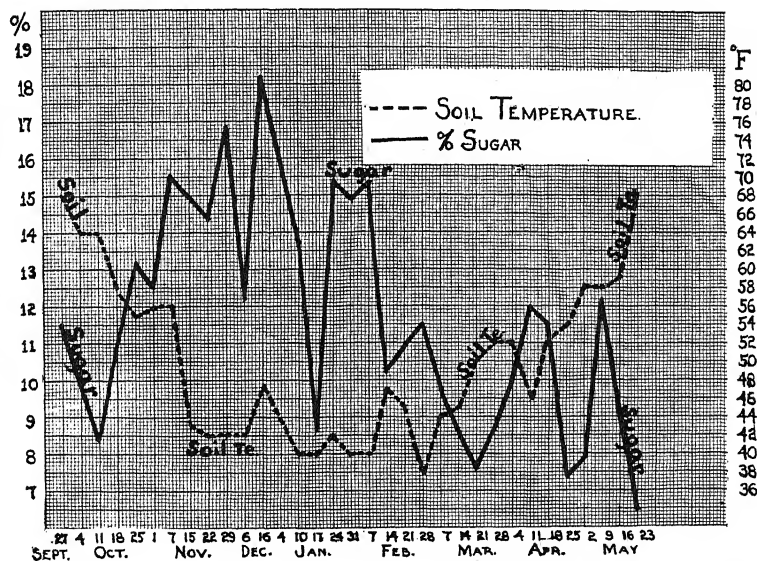


FIG. 1. Comparison of soil temperature with sugar concentrations of cell sap in alfalfa crowns.

divergent range. This is especially noticeable in Grimm, which seems to have a rather uniform concentration during the entire winter. Utah, with the greatest number of surviving ten-year plants, also has a comparatively small deviation. This observation is supported by the standard deviations from the mean of the several selections. GREATHOUSE and STUART (1) express their result with clovers: "... the adaptability of the red clover plant to winter conditions is closely associated with its metabolic rate." It is apparent that similar conclusions may be drawn in summarizing alfalfa.



During the last two weeks in May, similar determinations were made with a February, 1938, planting of five Minnesota varieties of alfalfa. Table II indicates that the sugar concentration does not differ materially from that of the older plantings.

TABLE II  
YOUNG ALFALFA MAY, 1938

	MINNESOTA GRIMM	LADAK	IDAHO COSSACK	DAKOTA NO. 12	MINNESOTA VARIEGATED
Sugar .....	% 10.2	% 7.6	% 7.6	% 8.7	% 9.2

A record of the soil temperature has been made for a number of years, and the averages for the winter of 1937-1938 are compared with the sugar concentrations of the alfalfa for the same period, in figure 1. It may be noted that November was an unusually cold month for Oklahoma. The sugar concentrations increased as the soil temperature dropped. A warm week during the first part of December was accompanied by a drop in sugar concentration. Rain in January also caused a decrease in sugar. During the spring months, the rise of temperature is followed by decreases in amounts of sugar.

### Summary

1. The hardier selections of alfalfa have more uniformly higher concentrations of sugar than the less hardy.
2. The concentration of sugar varies inversely as the soil temperature.

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BRIEF PAPERS  
RELATIONS OF PLANTS TO MINUTE DOSES OF  
INHIBITIVE SUBSTANCES

ERNEST S. REYNOLDS

It is generally recognized that minute doses of substances which are toxic in larger quantities may act in a stimulatory fashion, that is, they may cause the plant to accelerate one or more of its activities in excess of any stoichiometric relationship. Minute traces of mineral salts (2, 4) and auxins, bioses, vitamins, and other growth accessories (1, 3, 7) which are of biological origin, all seem to show this action and, above certain specific concentrations, the toxic action of representatives of most of these classes of materials has been demonstrated. The action of these substances, however, is much more complicated than the just mentioned facts would indicate. Living organisms not only react by stimulation or retardation of their individual activities, but also ultimately become accommodated to increased or decreased quantities of physical and chemical stimulative factors. This accommodation or acclimatization may become evident in various ways. THIMANN (6) has briefly called attention to a type of adjustment which is of very great significance in physiological studies. He says: "If roots are treated with auxin so that they are inhibited, and the auxin afterwards removed, their subsequent growth is accelerated. . . . The higher the concentration of auxin used, the longer it takes for this acceleration to appear, but on the whole the greater the acceleration is when it does come." Apparently referring to the same work WENT and THIMANN (8, p. 144) suggest that this reaction "presumably, is due to the rapid disappearance of auxin from the root, so that its concentration eventually reaches the accelerating level."

Some recent observations incidental to another investigation, upon rooting of oleander cuttings in solutions of hetero-auxin indicated the same type of reaction. Three sets of cuttings were made on April 18, some cuttings being placed in tap water (set C), some in 0.0013 per cent. hetero-auxin in water (set B), and some in 0.02 per cent. hetero-auxin in water (set A). Those in set B were given fresh solutions twice in the next three weeks, at the end of which time they were placed in tap water. They had developed callus tissue, but no root initials were externally visible. On April 20 the cuttings of set A were washed off and placed in tap water. The first cutting to show root development was from set C; and by May 9, 9 out of the 10 in this set had developed roots. On the other hand, by this date all of the cuttings of sets A and B had developed more callus and overgrowth of tissue than those in set C, but no roots. Those of set A had the greatest amount of overgrowth. By May 16 numerous roots were showing on all 3 cuttings in

set A, but in set B on only one cutting had a root appeared. In general the number of roots formed on the cuttings of check set C was less than on those of set A. By June 1, 6 of the 12 cuttings in set B had developed roots while the others had not rooted by June 14 and were discarded. Thus it is evident that the hetero-auxin stimulated tissue growth of the stem, and retarded root elongation. On the other hand more root initials were formed in the auxin-treated cuttings than in the non-treated, but at a considerably later date. The cuttings in the dilute solution which had been kept in this solution for three weeks were much retarded in root formation and suffered otherwise since half of them died during the experiment. Of those in this set which survived, several produced very large well-developed root systems, and some of the control cuttings likewise finally developed root systems which seemed the equal of some of the auxin-treated cuttings. Since these cuttings were used in another investigation, exact quantitative results can not be given.

The internal mechanism by which this type of adjustment takes place is of course a part of the irritable quality of protoplasm, but probably in each instance the detailed reactions are specific and different. In the reporting of experimental results and in making deductions from them, this type of reaction seems to have been given little if any consideration. It seems probable to the writer that this same type of adjustment would be likely to take place whenever there is a quantitative change in any of the stimulative factors of the environment, within the life-limits of the given factor.

The writer (5) formerly called attention to an accommodation of plant growth to a toxic substance in the case of a fungus, *Fusarium lini*, under the action of KCN. The initial growth of the fungus was retarded and the later growth accelerated, roughly in direct ratio to the increased concentration of the cyanide. More recently a preliminary inhibiting action followed by stimulation has been reported by WRIGHT and ANDERSON (9), in the action of water soluble derivatives of 1, 2, 5, 6-dibenzanthracene on the growth and glucose utilization of *Fusarium lini*.

WENT and THIMANN (8, p. 143) state that the inhibition of roots by auxins "is not comparable with that produced by toxic substances" but they do not elaborate upon this. The inhibition and later stimulation of *Fusarium lini* by KCN seems to be similar, at least in certain aspects, to this root reaction to auxin. Presumably more of the KCN remained present in the cultures in which the highest concentration had been used than in the lower concentrations, while in the auxin treatment of the cuttings the external auxin solution was completely removed before stimulation appeared. This might not have been necessary if a more dilute solution had been used since other experimental results show that the auxin is stimulative in much more dilute solutions than were used. It is possible that above the range of

immediately stimulative concentrations and below the completely inhibitive ones there would be a range of concentrations in which the roots would become adjusted and finally stimulated.

In any case, this preliminary inhibition and final stimulation of plant tissues by toxic substances, thus indicating an accommodation of the organism to them, points to the necessity of a more careful consideration of this phenomenon in physiological experimentation.

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## CONDITION OF CHLOROPHYLL IN THE LEAF<sup>1</sup>

O. L. INMAN AND MARIE L. CROWELL

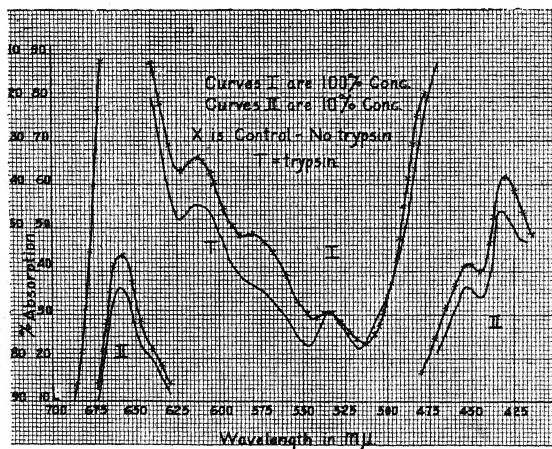
(WITH TWO FIGURES)

Extensive study of the microscopic structure of chloroplasts has been carried out by many workers. While this has given us considerable information about the probable nature of the condition of the chlorophyll pigments in the chloroplast there is still much to be explained.

The present experiments were undertaken for the purpose of investigating the physical and chemical state of the chlorophylls in the leaf.

*Trifolium repens* leaves were ground with sand, extracted with buffer solution, filtered, and centrifuged so that only the colloidal material was retained as the triturate. Using this triturate it is easy to recognize when the chlorophylls are losing the magnesium from the molecule: first, by the proportional increase of the intensity of the 534 m $\mu$  absorption band (ether as solvent) as compared with the other bands; and later, by the shift of the 662 m $\mu$  band toward the red end and appearance of a typical pheophytin spectrum.

Figure 1<sup>2</sup> shows the effect of adding 0.15 gm. of a crude trypsin (Eimer



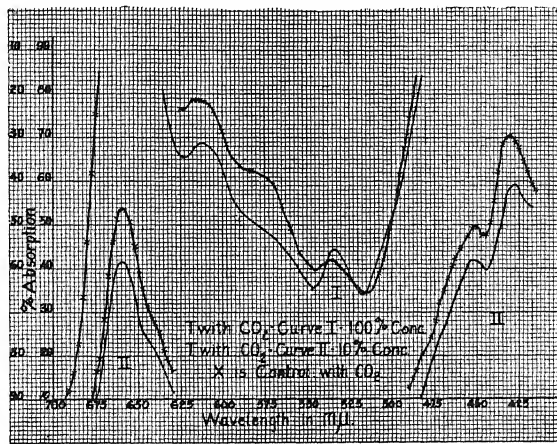
and Amend B 54) to 75 ml. of triturate and letting stand for 12 hours at 25° C. After the trypsin acted, the triturate was extracted with acetone and the chlorophylls transferred to ether by means of water. It is clear that the intensity of the 534 m $\mu$  band is proportionately greater with the use of trypsin.

<sup>1</sup> From the C. F. KETTERING Foundation for the Study of Chlorophyll and Photosynthesis.

<sup>2</sup> The curves for figures 1 and 2 were made by Dr. V. M. ALBERS (ALBERS, V. M. A recording photoelectric spectroradiometer. Jour. Opt. Soc. Am. 28: 121-123. 1938.).

The use of 0.0375 gm. and 0.075 gm. of trypsin clearly indicated that the degree of intensity of the 534  $m\mu$  absorption band was dependent upon the amount of trypsin added if the time, temperature, pH, and the amount of the triturate remained constant.

Figure 2 shows the same experiment except that carbon dioxide was



bubbled through the tritirates one hour before the chlorophylls were extracted. It is evident that the 534  $m\mu$  absorption band in the triturate containing trypsin is proportionately more intense than the control after the passage of carbon dioxide into the tritirates. The pH of the tritirates at the beginning of the experiment was 5.6 (measured with a glass electrode) and remained about 5.6 in both the control and the one to which trypsin had been added. After bubbling carbon dioxide through the control and the trypsin-containing triturate the pH was 5.0. An attempt was made to bring about the same increase in intensity of the 534  $m\mu$  band in the control by bubbling carbon dioxide through the triturate three hours. This further addition of carbon dioxide from a tank did not produce detectable change in the intensity of the 534  $m\mu$  band or bring the pH lower than 5. These experiments were repeated with NORTHROP's crystalline trypsin and gave the same kind of results. Similar results were also obtained when tritirates with pH 6.8 were used as starting material. In this case the pH after adding trypsin and without trypsin was 6.4, and yet the 534  $m\mu$  absorption band became more intense in the triturate where trypsin was used.

It seems fair to conclude from these experiments that trypsin causes the Mg to be freed from the chlorophyll molecules with the formation of pheophytin, and makes the chlorophyll molecules of the triturate more susceptible to the displacement of the Mg by means of the addition of carbon dioxide. It is also clear that it is not just a matter of the change in the pH of the triturate

since that would naturally form pheophytin if kept too far on the acid side of neutrality for a period of time such as twelve hours at 25° C. It must also be clearly understood that no pheophytin formation has been observed when the triturate is definitely alkaline.

One might propose several explanations for these reactions. It appears that the action of trypsin on the substrate, to which the chlorophylls must be closely bound by adsorption or chemical combination, is acted on by trypsin, and the hydrogen-ion concentration of some allied substance is so altered that pheophytin begins to form; yet no lower pH is recorded. The most obvious substrate to be thought of is a protein. If this is true it may be a good assumption to consider the chlorophyll pigments as being bound to their substrate through a Mg-protein linkage. There is, however, another assumption which is being tested: will the trypsin not hasten the release of Mg from pure colloidal chlorophyll by acting directly on the magnesium-nitrogen linkages in the chlorophyll molecule? If this were true, then the assumption of a linkage between chlorophyll and a substrate native protein would not be so obvious.

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## AN AUTOMATIC PLANT IRRIGATOR AND RECORDER

C. N. JOHNSTON AND O. A. ATKINS

(WITH ONE FIGURE)

Many forms of automatic irrigating equipment have been used by investigators growing plants in small containers. Most of these units have been devised to keep the gross weight of plant and container constant. To achieve this, water is added to the soil in small amounts as the plant consumes it. As a result the plant is often assumed to be growing under specified soil-moisture conditions but the facts contradict the presumption because it has been shown<sup>1</sup> that water added in this manner usually wets but a limited portion of the soil in the container to field capacity. The rest of the soil in the container will be much drier than the field capacity. The apparatus described below will automatically supply the soil mass within the pot with sufficient water to bring all the soil contained therein to the field capacity. In addition, a counter operated by the mechanism records the number of irrigations applied.

Figure 1 shows the apparatus with a cotton plant in a 10-quart container. It is seen that the apparatus is constructed from material usually found about the laboratory. The operation of the equipment is as follows: As pictured, the plant may be presumed to have been irrigated recently. The pot (no. 9) is heavy and has raised the counterbalance weight A against the stop, a nut on a machine screw soldered on the strap-iron leg running horizontally to the left of the top of the pot. As the plant removes water by transpiration the pot becomes lighter and at a predetermined weight, the counterbalance A will outweigh the pot. When this occurs, the pot will be lifted slowly till the catch B, pictured on the top of the shaft C in front of the pot, is released. When this occurs the water container E on the vertical wood support D at the left tilts forward swinging about 30 degrees and pivoting on the copper tube located close to the bottom of the can, and slightly back or to the left of the center of the container. When E has tilted, the syphon hose running across its front becomes primed and the water held in the container is discharged rapidly into the pot which becomes heavy and sinks till the stop again holds it. With the almost complete emptying of E, its weight is reduced so that the weight holder F, fastened to an extension of the pivoting tube, outbalances it and swings it back into vertical position. When E is vertical, the pivoted catch B on the other end of the linkage re-engages to hold E upright till the next release by the pot, which will not occur till the plant has used the soil moisture down to the desired limit. The small shot weight container attached to shaft C aids in balancing the linkage between

<sup>1</sup> VEIHMEYER, F. J. Some factors affecting irrigation requirements of deciduous orchards. *Hilgardia* 2: 125-291. 1927.



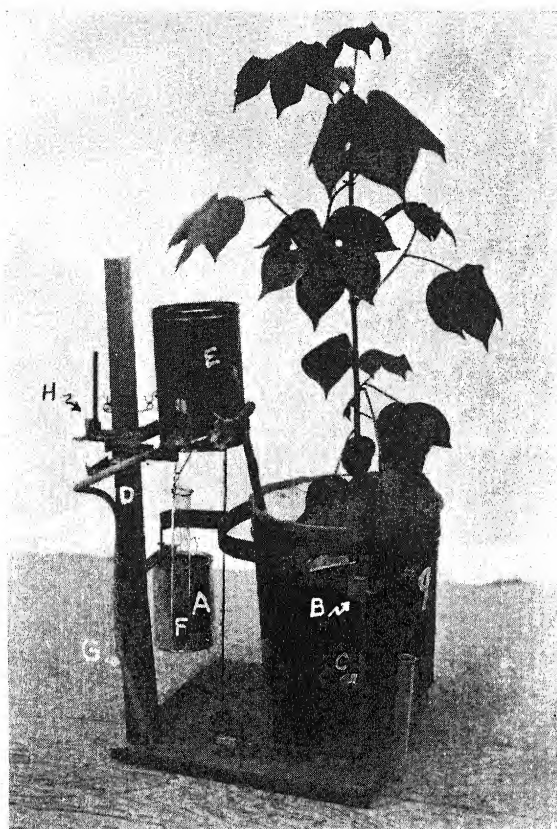


FIG. 1. Automatic irrigator and recorder with cotton plant.

the water container and the catch. The water supply to E comes from a constant level source such as a Mariotte flask. It enters the pivot pipe through the hose G running down the wooden shaft D and runs slowly through a 1/16-inch hole in the copper tube inside the can. The counter H is located on the stick supporting the water container and is operated by the motion of that can. As the can falls forward, the thick flat plate just below the copper shot tube to the left of the supporting stick is swung horizontally by the linkage and carries shot over to a hole in the plate below. This shot drops into the basket placed just underneath this plate. No shot escapes the shot tube during this motion, because the pivoting plate is wide enough to remain in sliding contact with the bottom of the tube. When the water container E is swung back to vertical, the moving plate on the counter is moved back so that the hole for the shot falls directly under the shot tube and one shot drops into position for the next cycle.

The correct volume of water to be added for each irrigation is regulated by moving the tilting water container E up or down on the supporting stick, keeping the lower edge of the syphon just above the required water level. Small adjustments in the height of the water container may be compensated at the bottom of the vertical link dropping from it. Changes larger than an inch require the use of different length vertical links. Otherwise all parts are standard. Hacksaw blades provided satisfactory pivoting surfaces for the operation of the balance system for the pot. They were soldered to the bracket supports. The ratio of loading of pot to counterbalance weight was 5 to 1, so the pivot arm lengths of pot and counterbalance can were in the ratio of  $1\frac{1}{2}$ " to  $7\frac{1}{2}$ ". While operating a large number of these irrigators, a carburetor float valve may be substituted for the Mariotte flask, using the domestic water supply as a source, with satisfactory results.

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## NOTES

**Summer Meeting.**—The summer meeting of the American Society of Plant Physiologists at Milwaukee, and at Madison, Wisconsin, will be held on June 20–22, 1939, in connection with the A.A.A.S. Registration headquarters will be in Milwaukee, and the first sessions will be held there. Other sessions have been arranged to be held at the University of Wisconsin. We hope that a large number of members and friends of the Society will be present on the opening day.

The headquarters will be at the Hotel Schroeder. In Madison, accommodations may be obtained at the Belmont Hotel and Cardinal Hotel at \$2.00 and up, and at the Park Hotel and Loraine Hotel at \$3.00 and up. Those who are driving cars to the meetings will find the large and well-appointed Monona Tourist Cabin Camp convenient. It is located on route 51, east of Madison, and may be approached from route 18 by a turn to the right, or from route 30 by a turn to the left, on reaching route 51 coming west from Milwaukee. Accommodations with breakfast will be available for a limited number in a lake-shore dormitory. If you desire a reservation, communicate with D. L. HALVERSON, Chadbourne Hall, Madison.

The program for Tuesday at Milwaukee presents a symposium on photoperiodism during the morning, and a general session for short papers in the afternoon. On Wednesday and Thursday at Madison invitation programs, inspection trips, and a picnic provide ample opportunity for scientific and social enjoyment. Come, and bring your friends along.

**Western Section.**—The fourth annual meeting of the Western Section of the American Society of Plant Physiologists will take place at Palo Alto, California, during the week of June 26 to July 1, 1939. The program will include three symposia: one in joint session with the Botanical Society on *Translocation of Solutes in Plants*, with T. G. MASON, Trinidad, British West Indies as chairman; one on *Growth* with F. W. WENT, California Institute of Technology as chairman; and one in joint session with the Western Society of Soil Science. Three half-day sessions are reserved for the reading of short papers.

In addition to the biologists' dinner on Wednesday night, there will be an informal dinner for plant physiologists on Friday evening. On Saturday morning there will be a trip to the laboratories and greenhouses of the Division of Plant Nutrition at Berkeley. Professor D. R. HOAGLAND has consented to conduct this trip, and it affords an excellent opportunity for visiting plant physiologists to see this outstanding laboratory.

Rooms and meals will be available on the Stanford campus. Those who

desire to avail themselves of the organized service for visitors may address Miss ANASTASIA DOYLE, Director of Residence for Women, Box 1772, Stanford University. Charges will be \$2.00 per day per person up to 3 days, and \$1.75 per day for 4 or more days.

**Changes in Proofs.**—The costs of making changes in the proofs of manuscripts are always large enough to be a considerable factor in the expenses of publication. The method of computing such costs is being changed to make possible the assessment of such costs against individual authors. Hitherto the charges have been made on an hourly basis, a method which did not facilitate computation of author responsibility. A new arrangement based upon the number of lines reset will make it easy to compute the changes in each paper. The charge is 12 cents per line for resetting galley and page proofs. We hope that authors will do at least an equivalent amount of thinking before they order changes made in their proofs. Actual errors must be corrected, of course; but the insertion of words, or omission of words, may cause many lines to be reset. If authors will retain the manuscripts until they are sure of just what they desire to say, if qualifying words and phrases are inserted or deleted in the manuscript before it is edited, most of the necessity for revision will be obviated.

**Drawings.**—Attention is again directed to the size of drawings sent with manuscripts. It is inadvisable to submit any drawings larger than the type-written page. If made larger, they should be reduced photographically before they are sent in for consideration. A clear photograph not over 4.5 inches in width will give the author an idea as to whether the lines, letters, and numerals are large enough for successful reproduction. Many drawings arrive in dog-eared and torn condition because they are made on paper larger than the manuscript and not properly protected from damage. If the drawings are to be submitted to readers, photostat copies should be included for that purpose, so that the originals may be safely preserved against loss and damage while being circulated to the readers of manuscripts. Cooperation on the part of all authors is requested.

**Errata.**—Several errors occurring in volumes 12 and 13 of PLANT PHYSIOLOGY have been reported, which seem to the editor important enough to need correction.

VOLUME 12, plate opposite p. 565, the date of birth of HANS MOLISCH should read December 6, 1856.

p. 216, line 6 from bottom, for "July 12, 1856" read December 6, 1856.

VOLUME 13, p. 394, line 2, for "(1 p.p.m.)" read (5 p.p.m.), and for "(0.15 p.p.m.)" read (0.75 p.p.m.).

p. 397, to tabulated optimal synthetic solution add:

MgSO<sub>4</sub> ..... 35.00 mg.

**Physical Methods Committee.**—The Physical Methods committee of the American Society of Plant Physiologists has been reorganized to continue the important work which has been undertaken by this committee. Occasional reports will be prepared covering fields where the need for more refinement of techniques, or better understanding of principles and practical difficulties, is greatest. The committee was first organized in 1930, and has contributed a number of papers in the interests of better science. The following contributions are noted: *Light Sources and Light Measurements*, by HARDY L. SHIRLEY, 1931; *Osmotic Quantities in Plant Cells in Given Phases*, by A. URSPRUNG, 1935; *Methods of Research on the Physical Properties of Protoplasm*, by W. E. SEIFRIZ, 1937; *Conductivity Measurements of Plant Sap*, by GLENN A. GREATHOUSE, 1938.

Professor R. B. WITHROW, Purdue University, is chairman of the committee, and the other members are K. C. HAMNER, University of Chicago; B. S. MEYER, Ohio State University; O. L. INMAN, Antioch College; and S. T. DEXTER, Michigan State College. EARL S. JOHNSTON, of the Smithsonian Institution, former chairman of the committee, will serve in an advisory capacity.

**Tree Growth.**—In this volume MACDOUGAL brings together the results of many years' work with his dendrographs and dendrometers, as well as a store of related information on the growth of numerous Californian and other trees. One cannot but marvel at the energy with which the veteran, now in his 74th year, continues his researches. The most detailed study is devoted to the species *Pinus radiata*, the Monterey pine. Progressive changes in diameter of trunk and roots at different points along the axis, initiation and cessation of cambial activity, height increment of stem and apical growth of roots, the effects of partial defoliation at different seasons—all are here. The author tries to correlate growth with the factors which might control it, such as temperature, water supply, and hormone production. In this connection some experimental data on water contents and further microscopical observations of cambium would have been more convincing than mere inference, but even MACDOUGAL cannot tackle all sides of the problem.

Dendrograph records are of fundamental importance in many phenomena of tree physiology. The daily fluctuations of diameter which they reveal have long been regarded as the best evidence in favor of DIXON's cohesion theory of sap ascent. With reference to the theory of hormone regulation of cambium action, the author's findings are "consonant with the conception of hormonal control of growth by auxins from the buds" as regards diametral growth of the trunk and perhaps of the proximal portion of the root; but they are opposed to such control of the distal portion of the latter in which cambial activity is more related to growth of root apices. Photosynthetic activity is

also illuminated by growth records considered in conjunction with leaf counts. The author has estimated the amount of wood formed per leaf of Monterey pine. One tree in its tenth year produced enough wood to form a layer 1.3 mm. thick over each leaf. The photosynthetic activity of a century old pine is estimated at 400 million leaf-years.

Of Californian superlatives the book has its share. There seems to be no doubt in the author's mind that the epithets oldest, largest, tallest, and fastest growing, all pertain to trees of the state. It is perhaps not surprising that *Pinus radiata*, his first love, to whose charms he has devoted so many years, is credited with forming cellulose at a higher rate than any other tree, but one wonders if BRADLEY'S *Albizia moluccana*, with a growth from seed to an average height of 86 feet and a diameter of 9.75 inches in 7 years,<sup>1</sup> or some of the new hybrid poplars are also in the running.

There is so much of interest in this book that one is disproportionately irritated by the few flaws that mar it. Many of the tables, particularly those (such as xxviii) dealing with dendrometer readings, could have been rendered much more intelligible. Actual errors are few and far between; the figure at the bottom of p. 90 should be 0.000025 mm.; the height level in larch (p. 86) is surely not 27.5 M. (92 ft.).—G.W.S.

The volume is published by the Chronica Botanica Co., Leiden, 1938, and contains vi + 240 pp. The price is 7 guilders.

**The World of Plant Life.**—An excellent book about plants that will delight the layman as well as the connoisseur botanist is Dr. CLARENCE J. HYLANDER'S *The World of Plant Life* which comes from the Macmillan Co., New York. Profusely illustrated with good drawings and unusual photographs, the book leaves little to desire for beauty and attractiveness. The author, moreover, has a gift for popular writing which we hope he will exercise in other ventures, for botany has too few worthy advocates before the bar of public opinion. Books of this type can do untold good in public education.

The order of presentation follows fairly conventional lines of evolutionary sequence. There are five parts devoted to: the thallus spore plants; the leafy spore plants; the naked-seed plants; the fruit-seeded dicots; and the fruit-seeded monocots. The author uses the technical terms for these sections of the plant kingdom, but in parentheses. Each part is subdivided into chapters dealing with smaller groups within the main sections, and the author presents the entire classification in 10 phyla. There are 46 chapters, and all of them give one thrilling excursions into the fields of knowledge. Take the chapter on the cacti, for instance. Every cactus enthusiast will enjoy the 25 pages of description and exquisite photographs and be hungry for more at the end.

<sup>1</sup> Indian Forest. 48: 637-640. 1922.

One could wish nothing better than that young students of botany might be given such fare as this along with their stodgy and insipid courses in botany. It would add such spice and flavor as to make the study of plants a genuine pleasure while leading to the important fields of investigation. Every school library should possess it, and individuals will prize it in their private libraries. The check list of plants according to phylum at the end of the work (48 pages) may be used by classes to keep a record of their field experiences. A selected bibliography and an index complete this unusual work. The editor feels that this book is an outstanding contribution to popular learning, and while it is somewhat expensive, \$7.50 per copy, it is worth a great deal more.

**Annual Review of Physiology.**—Our readers are familiar with the *Annual Review of Biochemistry* which has achieved an outstanding place among annual reviews. We take pleasure in introducing to our members the first number of *Annual Review of Physiology*, which has been edited by Dr. JAMES MURRAY LUCK and his colleague Dr. VICTOR E. HALL, both of Stanford University. The aim has been to attempt a critical appraisal of contemporary work in definite fields, to analyze and interpret the most significant contributions, rather than to include mention of all the papers appearing in physiological journals. Such reviewing, well done, assists in the integration of science, and is necessary to effective progress in physiological research. There are 24 reviews in this first volume: Permeability, by M. H. JACOBS; the biological effects of radiation, JANE HOWELL CLARK; physiological aspects of genetics, G. W. BEADLE; developmental physiology, JOSEPH NEEDHAM; growth, C. B. DAVENPORT, with OTTO RAHN, MARY E. MAVER, H. W. CHALKLEY, and D. M. PACE; temperature regulation, ALAN C. BURTON; energy metabolism, JOHN R. MURLIN; the peripheral circulation, H. C. BAZETT; respiration and its adjustments, ROBERT GESELL; muscle, EMIL BOZLER; the digestive system, A. C. IVY and JOHN S. GRAY; physiology of the liver, FRANK C. MANN and J. L. BOLLMAN; blood: physiology of formed elements and plasma; blood clotting, J. HAROLD AUSTIN; heart, J. A. E. EYSTER; electrical phenomena of the brain and spinal cord, HALLOWELL DAVIS; the spinal cord and reflex action, J. C. ECCLES; bioelectric studies of the excitation and response of nerve, DETLEY W. BRONK and FRANK BRINK, Jr.; the autonomic nervous system, JOSEPH C. HINSEY; the special senses, J. M. D. OLMSTED; physiological psychology: the conditioned reflex, ERNEST R. HILGARD, and miscellaneous topics, CALVIN P. STONE; kidney, HOMER W. SMITH; general and local anaesthesia, M. H. SEEVERS; applied physiology, D. B. DILL; and endocrine glands: gonads, pituitary, and adrenals, HERBERT M. EVANS.

Author and subject indexes supply means of rapid use of the material included. Plant physiologists who desire to maintain a well-balanced view



of the entire field of physiology will find it advantageous to browse the excellent material offered in these reviews. All of them bear the marks of conscientious and painstaking effort on the part of the reviewers. It is a fine companion series to the *Annual Review of Biochemistry*.

Copies may be purchased at \$5.00 each, from Annual Reviews, Inc., Stanford University P.O., California.

**Chemistry of the Amino Acids and Proteins.**—An unusually fine treatise on the amino acids and proteins has been brought out through the efforts of Dr. CARL L. A. SCHMIDT, University of California. Sixteen contributors cooperated in the preparation of the book, which covers this vast field very ably. The first 10 chapters deal with the chemical statics of the amino acids and proteins, and the last 8 with chemical dynamics. Although the various chapters have been written by different authors, the work as a whole has been carefully planned with logical sequence of the chapters. A short account of the history of discovery in these fields is followed by chapters on the constitution and synthesis of the amino acids; isolation of the amino acids from proteins; preparation of amino acids and proteins; methods of analysis and reactions; relation of the amino acids to products of biochemical importance; peptides, peptidases, and diketopiperazines; chemical constitution of the proteins; molecular weights of proteins; certain chemical and physical characteristics of the proteins; and optical properties of amino acids and proteins. The dynamics includes chapters on the amphoteric properties, electrochemistry, combining properties, membrane equilibria, thermodynamics, relations to immunity, and rôle of proteins in nutrition. One cannot overstate the value of such a comprehensive treatment of the amino acids and proteins. The work contains over 1000 pages, and may be obtained at \$7.50 per copy. Orders may be sent to the publisher, Charles C. Thomas, Springfield, Illinois. He deserves congratulations on having placed this valuable volume before the public.

# PLANT PHYSIOLOGY

JULY, 1939

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## ATMOSPHERIC HUMIDITY AND TEMPERATURE IN RELATION TO THE WATER SYSTEM OF PLANTS AND SOILS<sup>1</sup>

CHARLES A. SHULL

(WITH FOUR FIGURES)

### Introduction

From the very beginnings of plant physiology as an experimental science, the water relations of plants have received much attention. During two thousand years before VAN HELMONT's classic experiment with the willow shoot, Grecian thought had dominated the field of natural history. Plants were believed to receive from the earth, through their lacteal roots, a perfect pabulum from which their entire bodies could be constructed, with nothing needed for growth lacking, and, since there were no obvious excretions, nothing ever left over as waste. The earth was likened to a gigantic stomach within whose recesses the food of plants was digested and elaborated into a form corresponding exactly to the plant's needs. A single building substance was believed to be taken in through the roots, distributed through fibrous vessels to all parts of the plant body, and used in the complete construction and nourishment of all of its organs.

The first experiment in plant physiology is usually ascribed to VAN HELMONT, who was one of the most distinguished iatro-chemists of his period. When VAN HELMONT in 1610 concluded from his experiment that the 164 pounds, three ounces of dry weight of willow wood, bark, roots, and leaves had been constructed entirely from the rain or distilled water which he had supplied it, his conclusion was typically Grecian. He merely had proved to his own satisfaction that water alone was that perfect pabulum of plants. The fact that 2 ounces of earth was lost in some manner during the 5 years did not disturb him. If he thought at all about this loss of earth, he must have felt as we moderns are wont to do, that a loss of only slightly more than

<sup>1</sup> Fourth STEPHEN HALES address, read before the American Society of Plant Physiologists at St. Louis, December 31, 1935.

0.06 per cent. of the earth was insignificant in view of the fact that 164 pounds of dry matter had been formed.

This experiment was not left long unchallenged, however; the work of GLAUBER on salt petre in 1656, and the beautiful experiments of WOODWARD (28) on spearmints in 1699 fully confuted VAN HELMONT'S views. These spearmints were grown in water from different sources, and were found to flourish somewhat in proportion to the amount of earthy contamination which occurred in the water. Insisting that it is the earthy material itself that serves as the pabulum of plants, JETHRO TULL (26) remarks concerning the early "water philosophers," that they "were deceived, in not observing that water has always in its intervals a charge of earth, from which no art can free it." That VAN HELMONT should have drawn unwarranted conclusions from a well-planned, well-executed experiment is not to his discredit. It merely indicates the difficulty of original work at this early period, and it may also stand as a warning to all of us who in our own age share with him the frailty of being human and of occasionally making errors in the interpretation of our results.

The most important early investigator of water relations of plants was STEPHEN HALES (4), to whom we annually pay our tribute of respect and honor for his contributions in that field. His interesting quantitative measurements were many years in advance of the period in which he lived. They were made long before osmotic phenomena had been discovered and rationalized in terms of plant behavior; before either hydrogen or oxygen had been isolated as elements; more than 50 years before CAVENDISH announced to the world the synthesis of water from its two constituent elements, thus proving its constitution; more than a hundred years before the establishment of the cell generalization by SCHLEIDEN and SCHWANN; more than a hundred years before the description and recognition of protoplasm as the fundamental living substance of living plants and animals. Many of HALES'S experiments are worthy of attention today. He measured the amount of water evaporated from the leaves of plants, measured the areas of root systems and total leaf areas of plants, from which he calculated the thickness of the layer of water absorbed by the root system in a 12-hour period, and the thickness of the layer of water given off by the leaves during the same time. From these studies he estimated in a crude way the rate of sap rise. His approach to this problem was the same as that recently used by CRAFTS (2) in his early attempts to account for phloem transfer of organic foods in cell walls. HALES compared the transpiration rate during the day with the rate at night, using methods that would make beautiful laboratory demonstrations for our classes today. He compared water loss from leafy and leafless twigs, and from deciduous and evergreen trees. He calculated the amount of water given off by an acre of hop vines in terms of a layer of water over the entire area. He studied root pressure (and also

blood pressure of animals) and made the first use of mercury manometers on decapitated branches of grape vines to measure their root pressures. He demonstrated the suction power of evaporation not only by means of severed branches, but by means of the roots of living trees. He captured and condensed the vapor exuded by leaves to determine whether it differed in any way from ordinary water. He investigated the possibility of translocation of odoriferous solutes through the stems to leaves and fruits. He proved that sap rise continues during winter. He measured changes in soil humidity and temperature through the seasons, and attempted to estimate the value of dew in the water economy of plants. He compared rainfall quantitatively with transpiration. He attempted to measure the imbibitional swelling of dry peas, and carried on a series of ringing experiments designed to show whether or not there is a circulation of sap in plants. He concluded that there is no real circulation of the sap, although he admits that sap can pass down the stems under certain conditions. He also made use of an instrument which he attached to grape vines to measure diametral contraction and expansion of their stems with water loss and water gain respectively. With a sensitivity of 0.01 inch, he was able to observe such expansion of vine stems during rains. These observations were the forerunner of modern dendrograph records.

It is not difficult to find defects in his work, inept reasoning, faulty interpretation, and lack of understanding of the plant body; but these contributions, considered with reference to the general state of knowledge in the early 18th century, form an unique and remarkable foundation for the great advancement which has been made since his time. The expanding literature on water relations attests year after year the perennial interest in these problems. The reason for this interest lies in the realization that there is no more fundamental relation than that of the active organism to its internal and external water supply. During the last 60 years we have seen the problems of imbibition and osmotic behavior analyzed in considerable detail as they relate to cellular physiology and to the life of the plant as a whole. Methods of measuring these relations with a fair degree of accuracy have been developed. We have seen also the gradual development of a consistent hypothesis to account for the ascent of sap in plants. The cohesion theory is at once mentally satisfying and challenging as a working hypothesis. At the same time, it must be considered only an hypothesis, and must be subjected to testing in every possible manner. We are also coming to see and to understand more fully the basic importance of relative humidity and temperature of the atmosphere in relation to the water system of plants and soils. It is the purpose of this discussion to focus attention upon the fundamental causes of changes in these water systems as these changes relate to the physiology of the organism.

### Forces of evaporation

It is now generally recognized that evaporation of water from the exposed aerial portion of plants is an unavoidable consequence of the differences in magnitude between the vapor pressure of the moist biocolloids of the plant body and of the vapor pressure of the surrounding atmosphere. The molecules of water held on the surfaces of these biocolloids are kinetically active, and some of them move with sufficient momentum to break loose from the surface attractions and diffuse into the atmosphere. How powerful are these movements? What force must be applied to the molecules to inhibit their escape, or to bring about the state known as dynamic equilibrium? The magnitude of the force required to establish such water equilibrium depends upon the condition of the atmosphere itself. In a nearly saturated atmosphere, the force required is very small, while in an extremely dry atmosphere the force required is very large. An attempt will be made to clarify this problem.

The tendency of water to escape from the colloidal cellular substrate into the environment may be reduced in several ways: (1) Lowering the temperature of the leaf decreases the kinetic motion of the water molecules, so that their momentum does not so frequently tear them loose; moreover, it reduces the water vapor tension of the internal atmosphere of the leaf, thus decreasing the vapor pressure gradient which carries the vapor away from the colloidal surfaces by diffusion. (2) Increasing the relative humidity of the air decreases the outward diffusion of water also, by reducing the vapor pressure difference or gradient of diffusion. (3) Increasing the internal forces, either of the water itself (as by increase of hydrated solute constituents), or of the surface attractions of the biocolloids for water, whether by chemical modification of the colloid or by mere drying, causes greater resistance to the kinetic action of the water molecules.

As has been shown in a recent paper (7), it is possible to calculate the force which must be applied to the water molecules to balance their escaping tendency from the colloidal surfaces, or to bring them into dynamic equilibrium with their environment. This force varies directly with the water vapor deficit<sup>2</sup> and temperature of the surrounding air. For every combination of these two factors there is a corresponding colloidal condition in which the forces of water movement are in equilibrium. The colloidal state of dryness at this point is a matter of empirical test; one must determine it by

<sup>2</sup> In table I the humidity relations are expressed in the usual manner, as percentage of saturation; it should be noted, however, that in the equation used for calculations,  $P_v$  represents saturation (100 per cent. relative humidity), and  $P'_v$  is the actually observed relative humidity, determined empirically for some definite time and place, *e.g.*, 80 per cent. relative humidity.  $P_v - P'_v$  is thus an expression of saturation deficit of the atmosphere, and in a calculation for this particular degree of saturation deficit (20 per cent.) becomes  $\log 100 - \log 80$ , or  $2.00000 - 1.90309 = 0.09691$ .

measurement; but the *forces* at equilibrium are calculable, and do not need to be measured.

The thermodynamic equation used in making such calculations was first used more than 50 years ago, but it has not found use in connection with the interpretation of water relations of plants and soils until recently. The equation is easily derived, and in the integrated form has the following expression:  $\Delta P_1 = \frac{RT}{V_1} \cdot \ln \frac{P_v}{P'_v}$ , which for purposes of calculation may be

expressed in the Briggsian system as  $2.3026 \cdot \frac{RT}{V_1} \cdot \log \frac{P_v}{P'_v}$ . The effectiveness of the environment in providing a partial water vacuum into which evaporation from living plants may occur is indicated in table I. The forces which would exist on the biocolloids and in the atmosphere at dynamic equilibrium have been calculated for sixteen different degrees of relative humidity (saturation deficit), and six different temperature values. These humidities and temperatures have been chosen to cover approximately the entire range of conditions presented by the natural environment of our land flora. Relative humidity seldom goes below 10 per cent., except possibly in the driest desert regions of the earth; and the biokinetic range of temperature for ordinary land plants may be considered as lying between 0° C. and 50° C. Seeds and spores in the dry state can withstand the lowest temperatures that man can produce, within a degree or so of absolute zero, without loss of life (9); but the temperature for active growth usually lies between the limits stated. The values presented in table I have been subjected to a minor correction<sup>3</sup> for the changing values of the molal volume of water with changing temperatures. For purposes of theoretical discussion the figures are, therefore, sufficiently accurate. They give an illuminating picture of the water conditions of the atmosphere throughout the range of relative humidity and temperatures to which living organisms are normally exposed.

In earlier papers (17, 18, 19, 21) the conclusion had been reached that dry seeds, when in dynamic equilibrium with their environment, were holding their hygroscopic water with a force of from 950 to 1350 atmospheres. From table I it may be seen at a glance that the corresponding humidities of the atmosphere which would generate forces with such values, would be in the neighborhood of 40 to 50 per cent. relative humidity. This is not an unusual value for the humidity of laboratory air, which is usually much drier than the outside atmosphere.

<sup>3</sup> The values of  $V_1$  used in the calculations are as follows:

0° C., $18 \times 1.00013$	30° C., $18 \times 1.00436$
10° C., $18 \times 1.00027$	40° C., $18 \times 1.00782$
20° C., $18 \times 1.00177$	50° C., $18 \times 1.01207$

These values were obtained from HODGMAN'S Handbook of chemistry and physics, 19th ed., p. 1115, 1934.

TABLE I

TENSIONAL FORCES REQUIRED TO STOP EVAPORATION (DYNAMIC EQUILIBRIUM) EXPRESSED IN ATMOSPHERES FOR VARIOUS RELATIVE HUMIDITIES AND TEMPERATURES THROUGHOUT THE BIOKINETIC RANGE

RELATIVE HUMIDITY	TEMPERATURE RANGE, °C.					
	0°	10°	20°	30°	40°	50°
%	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>	<i>atm.</i>
10 .....	2865.4	2969.9	3070.3	3166.9	3260.1	3350.2
20 .....	2002.8	2075.9	2146.0	2213.6	2278.7	2341.7
30 .....	1498.25	1552.9	1605.4	1655.9	1704.7	1751.7
40 .....	1140.25	1181.9	1221.8	1260.2	1297.3	1332.6
50 .....	862.6	894.0	924.2	953.3	981.4	1008.5
60 .....	635.7	658.9	681.1	702.6	723.26	743.2
70 .....	443.8	460.1	475.6	485.0	505.0	518.9
80 .....	277.7	287.8	297.5	306.9	315.9	324.7
90 .....	131.1	135.9	140.5	144.9	149.2	153.3
95 .....	63.8	66.2	68.4	70.56	72.6	74.6
96 .....	50.8	52.7	54.4	56.1	57.8	59.4
97 .....	37.9	39.3	40.6	41.9	43.1	44.3
98 .....	25.1	26.05	26.93	27.8	28.6	29.4
99 .....	12.49	12.95	13.39	13.8	14.2	14.6
99.5 .....	6.25	6.47	6.69	6.9	7.1	7.3
99.9 .....	1.23	1.28	1.32	1.36	1.4	1.44

The data of table I reveal that vapor pressure deficit is a much more important factor in determining the final value of the equilibrium condition than is temperature. The influence of a rise in temperature in creating greater equilibrium forces is seen by reading the lines of figures from left to right across the table. At a relative humidity of 95 per cent. (deficit 5 per cent.), a 10° rise in temperature increases the forces of dynamic equilibrium by the small amount of 2 to 2.4 atmospheres; and at 99.9 per cent. relative humidity, even a 50° rise in temperature raises the force at dynamic equilibrium by only 0.21 atm. At low humidities, however, the temperature effect is very much greater, a difference of 10° at 10 per cent. relative humidity increasing the forces at equilibrium by 90 to 100 atmospheres.

It is easier to visualize the fundamental difference in the magnitude of the effects of humidity (deficit) and temperature from graphs of the data in table I. In figure 1 the data are plotted to show changes in the equilibrium forces with temperature change. The increasing steepness of slope of the lines toward the top of the graph shows the increasing effects of temperature as the humidity decreases. These sloping lines, if projected to the left, will converge at absolute zero, approximately. The change in slope is just what would be obtained if the plotted distance from -273° C. to 50° C. were to be used as the radius of a circle which rotated through the range of naturally occurring humidities.

Still more striking are the curves of figure 2, which show the rapid in-

crease in the equilibrium forces with decreasing humidity (increasing vapor pressure deficit). The lower curve in this graph represents the changes with decreasing relative humidity when the temperature is maintained at zero; and the upper curve is plotted for the same changes in humidity with a maintained temperature of 50° C. The curves for 10°, 20°, 30°, and 40° C. would lie between those shown in the graph, and would pass, respectively, through the four points indicated between the curves shown. The tempera-

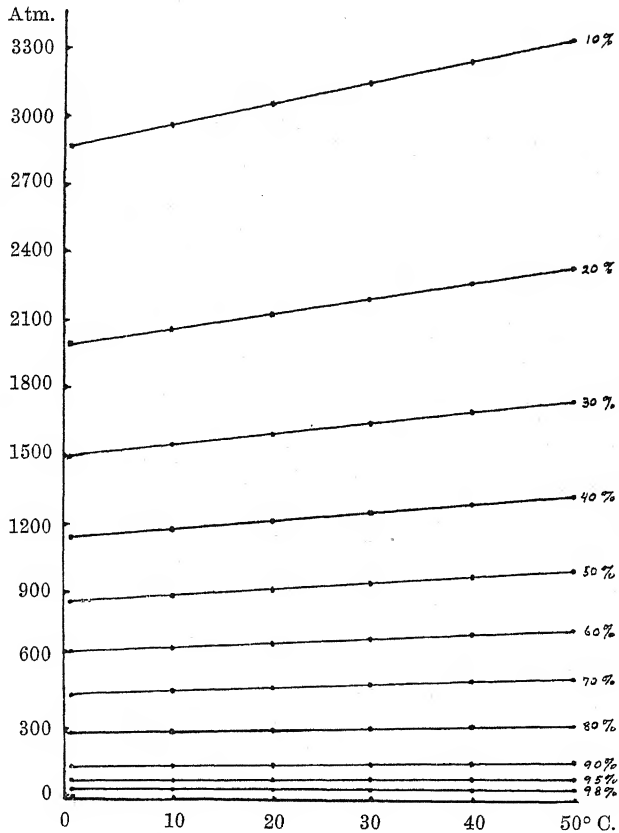


FIG. 1. Increases in the equilibrium forces with temperature increases.

ture influence is indicated by the vertical distance between homologous points on the two curves. The vertical distance between the curves becomes much greater with low humidities than with high. It should be clear from this graphic representation of the data that, of the two factors under consideration, the vapor pressure deficit is much more important than temperature in determining the character of the atmosphere with reference to water equilibrium conditions.



Wind flow is a factor in determining the *rate* at which the system will approach equilibrium, mainly because it sweeps moisture away from the leaf as rapidly as it diffuses through the open stomata. This brings the full

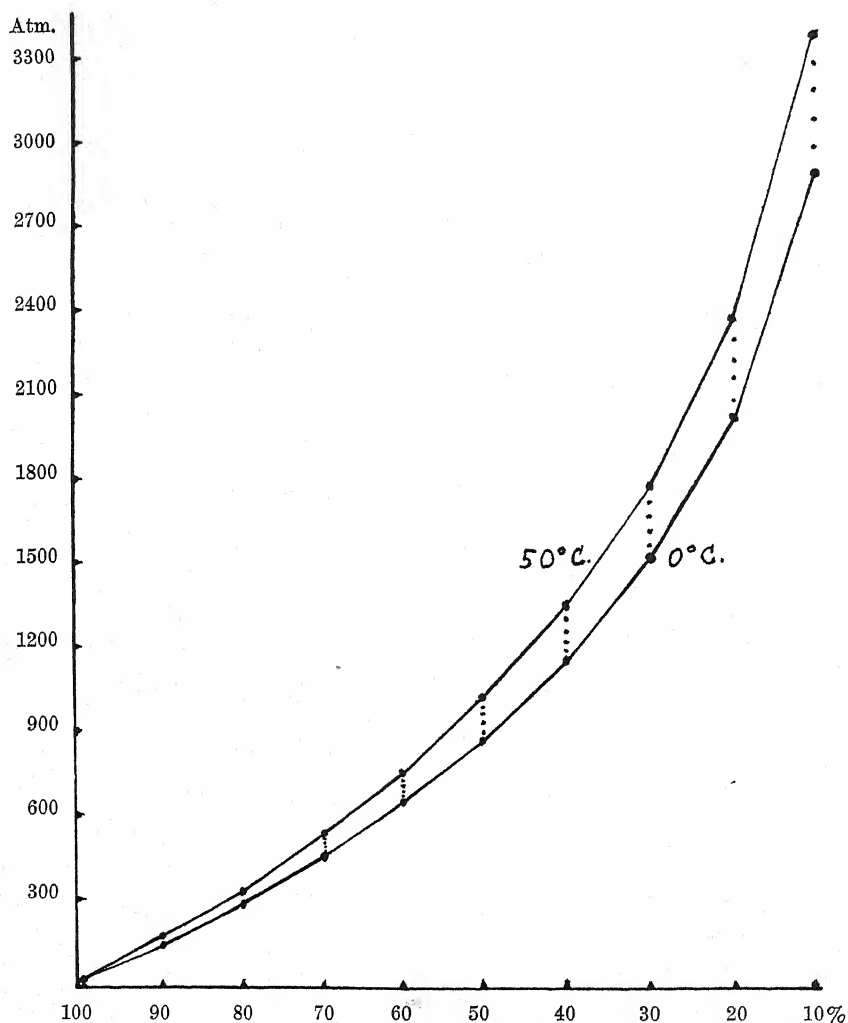


FIG. 2. Increases in the equilibrium forces with increasing atmospheric saturation deficit.

effects of atmospheric aridity to the surface of the guard cells, and into contact with the cutinized surfaces of the epidermal cells. MARTIN and CLEMENTS (10) have just shown that air flow of 1 mile per hour, approximately 1.5 ft. per second, causes a 30 per cent. increase in the transpiration of young sunflowers. Increasing the air flow to 16 miles per hour did not

suffice to double this effect. These results show that when the air flows rapidly enough to remove the diffusing water, additional air flow has only slight effect until stomatal closure is induced, which MARTIN and CLEMENTS found to occur at an air flow of about 5 miles per hour in the case of *Helianthus annuus*.

The atmosphere constantly fluctuates in relative humidity and temperature, with the result that the conditions for water equilibrium between the plant and its environment are constantly changing. Sometimes the changes are very large in a very brief period. To illustrate the changing conditions in concrete fashion, the U. S. Weather Bureau data from Washington, D. C., for 10 days in June, 1932, have been used as a basis for calculating the equilibrium water relations in atmospheres. Table II shows the combinations

TABLE II

DIURNAL FLUCTUATIONS OF EQUILIBRIUM FORCES AT WASHINGTON, D. C.,  
JUNE, 1932

DATE		RELATIVE HUMIDITY	TEMPERATURE	EQUILIBRIUM
		%	°C.	atm.
6/16/1932	8: 00 A.M.	94	21	83
	Noon	78	24	336
	8: 00 P.M.	90	21	141
6/17	8: 00 A.M.	84	21	233
	Noon	56	27	790
	8: 00 P.M.	77	25	354
6/18	8: 00 A.M.	81	19	280
	Noon	80	18	296
	8: 00 P.M.	86	17	199
6/19	8: 00 A.M.	94	17	82
	Noon	81	19	280
	8: 00 P.M.	85	19	216
6/20	8: 00 A.M.	92	18	110
	Noon	76	21	367
	8: 00 P.M.	80	22	299
6/21	8: 00 A.M.	78	22	333
	Noon	68	27	526
	8: 00 P.M.	74	27	410
6/22	8: 00 A.M.	66	26	565
	Noon	50	30	953
	8: 00 P.M.	53	30	873
6/23	8: 00 A.M.	53	26	862
	Noon	45	22	1072
	8: 00 P.M.	39	25	1275
6/24	8: 00 A.M.	38	17	1278
	Noon	24	21	1909
	8: 00 P.M.	29	25	1677
6/25	8: 00 A.M.	67	21	536
	Noon	32	29	1562
	8: 00 P.M.	40	29	1256

of relative humidity and temperature at 8:00 A.M., noon, and 8:00 P.M. from June 16 to June 25 inclusive, and the internal tensions which would exist in the plant at dynamic water equilibrium with the air under these conditions are shown in the last column of table II. The fluctuations are depicted in figure 3, which shows that equilibrium conditions are usually

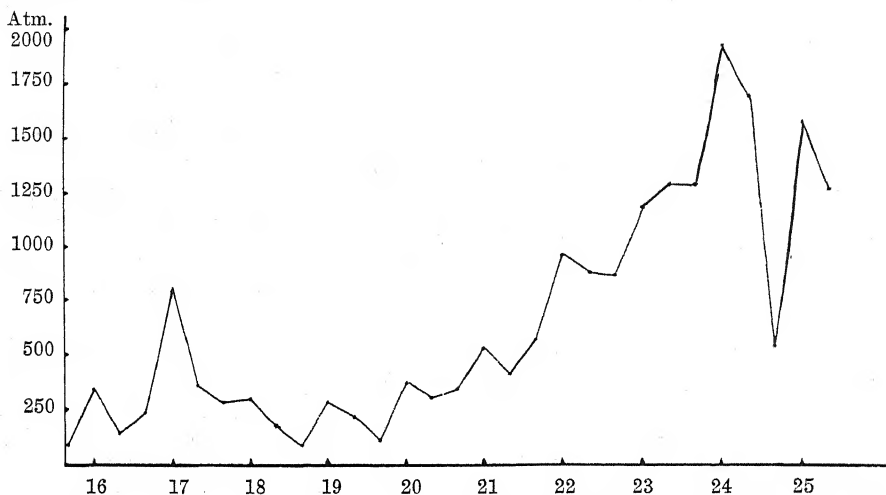


FIG. 3. Diurnal fluctuations of the equilibrium forces with diurnal changes in temperature and atmospheric saturation deficit. Data for Washington, D. C., June, 1932.

much higher in the middle of the day than in the morning or evening. This fact is generally recognized, but no precise quantitative expression of the fact has ever been given.

It is a well known fact, also, that laboratory air is drier than the natural atmosphere, but aside from humidograph or hygrometer readings, the differences between the equilibrium conditions of inside and outside air have never been given quantitative expression. A number of readings were made with a sling psychrometer, first in the laboratory, then in the natural atmosphere. Using MARVIN's psychrometric tables (11) the relative humidity was obtained in each locality. From the humidity readings and the dry bulb temperatures, calculations of the water equilibrium forces were made. Three laboratory readings were 1844, 1532, 1904 atm., respectively, while the corresponding readings outside were 484, 362 and 488 atm. The atmosphere in an oven room was yet much drier than the ordinary laboratory air, a single reading showing an equilibrium tension of 2271 atm. Measured in terms of the equilibrium forces in atmospheres, the laboratory air is about four times, and the oven room air almost five times, as severe as the outside atmosphere.

Organic and inorganic colloidal materials undergo fluctuations in mois-

ture content as the atmospheric conditions change, always toward the establishment of an equilibrium of forces. Seeds which are already in dynamic water equilibrium with the aqueous vapor phase of their environment will begin to increase in weight as soon as the atmosphere increases in relative water content or decreases in temperature; or they will lose weight if the

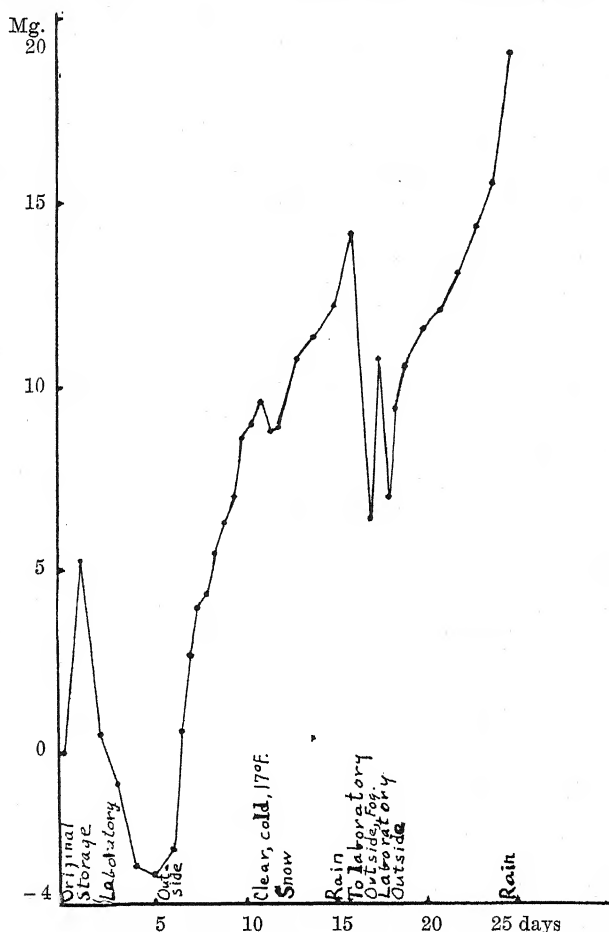


FIG. 4. Fluctuations in weight of organic colloids exposed to changing atmospheric conditions.

air becomes drier or warmer. Figure 4 shows some of the changes in weight of seeds of the Chisholm variety of field corn while subjected to changed environmental conditions. These seeds had been weighed individually some months before they were used in these experiments. At the beginning of the tests, the seeds were about 5 mg. per seed heavier than when first weighed. The preceding weather had been cloudy and rainy, but the seeds were stored

in a laboratory locker. Exposed in the laboratory for a few days, they lost weight. They were then placed out of doors in containers protected from precipitation. The plotted curve indicates the increasing weight as the seeds adjusted themselves to the outside conditions. After some days they were brought into the laboratory for 24 hours, then placed outside for 12 hours, again inside for 12 hours, then outside until they began to fluctuate with the natural environmental changes. It required considerable time for this adjustment, and only the earlier readings are shown in figure 4. It is obvious from this experience that the time lag is important in such work. The rate of diffusional flow of moisture into or out of the seeds must be somewhat proportional to the difference between the vapor pressure of the seed colloids and that of the atmosphere. All kinds of colloidal materials, including soil colloids, behave in this manner. These facts have long been recognized, but it has now become possible to measure and state the equilibrium effects with satisfactory precision.

To demonstrate the practical usefulness of the method, some seeds and some soils were confined in desiccators maintained at a controlled temperature of 20° C., over 4 different concentrations of sulphuric acid, the vapor pressures of which were known. Only an example or two are presented, as more work must be done, especially with the soils, before satisfactory conclusions may be drawn. The concentrations of sulphuric acid solutions used were as follows:

36.5 per cent. sulphuric acid, r.h. 64 per cent., at 20° C. = 595 atm.

50.0 per cent. acid, r.h. 37 per cent., at 20° C. = 1326 atm.

61.75 per cent. acid, r.h. 16 per cent., at 20° C. = 2444 atm.

76.3 per cent. acid, r.h. 3.8 per cent., at 20° C. = 4360 atm.

Marquis spring wheat exposed near the surface of the sulphuric acid for 28 days was found to contain the following percentages of moisture:

Over 36.5 per cent. sulphuric acid, 13.7 per cent. moisture.

Over 50.0 per cent. acid, 9.69 per cent. moisture.

Over 61.75 per cent. acid, 5.89 per cent. moisture.

Over 76.3 per cent. acid, 3.93 per cent. moisture.

Granted that the seeds had attained moisture equilibrium with the acid, one could then say that the force with which the 13.7 per cent. of water is held by the seeds is 595 atmospheres; with further drying, the force increases, and at a water content of 9.69 per cent., the remaining moisture is held with a force of 1326 atmospheres. The grains which have been dried down to a water content of 5.89 per cent., hold their moisture with a force of 2444 atmospheres; and those which had been reduced to 3.93 per cent. water content were holding this small quantity with a force only a little short of 4400 atmospheres.

The results with the soils were not very satisfactory, presumably because

the sassafras series of soils did not vary sufficiently in texture and composition to permit accurate readings in this preliminary attempt. Best results would surely be attainable with soils of higher colloidal content, with greater hygroscopic capacity. At equilibrium over 36.5 per cent. sulphuric acid a sample of sassafras loam contained only 0.76 per cent. of hygroscopic water, which was held by a force of 595 atmospheres; at equilibrium with 50 per cent. acid, the water content of this loam was reduced to 0.47 per cent., which was held by a force of 1326 atm.; at equilibrium with 76.3 per cent. acid only 0.26 per cent. of hygroscopic moisture was left, but it was held with a force of almost 4400 atmospheres. Other tests are planned with heavier soils from the black belt of Alabama. The most difficult problem in connection with such work is to make certain that equilibrium has been actually attained. In this work readings were taken at the end of a 28-day period, chosen arbitrarily, just as in earlier work on soil moisture (19) a 15-day period was chosen as appropriate for the attainment of equilibrium relations between soils of different moisture contents and dry seeds which were used for measurement of the resident surface forces of the soils.

#### Soil and plant responses to the aerial environment

By virtue of the relative humidity and temperature relations which have just been outlined, the atmosphere exerts a profound influence upon the water systems of plants and the soils in which they are rooted. Time will permit but brief reference to some of the more important considerations. In regions where extreme drought prevails during long periods, we see the deserts with their peculiarly adapted vegetation; and any region which suffers prolonged rainless seasons moves rapidly toward desert conditions, a fact painfully illustrated over large areas of the great plains region in recent years.

Soils lose their water by gravitational drainage of free water, by direct evaporation from the soil surface, and by indirect evaporation through the exposed portions of the ground cover vegetation. As long as the soil is well supplied with freely movable capillary water, severe transpiration may take place locally and temporarily without doing much harm to the vegetation, and without producing drought conditions. No land area is ever very far away from drought conditions if the atmosphere is dry and warm for prolonged periods. It was observed in Kansas (20) some years ago that when the atmosphere becomes so arid that the evaporation consistently exceeds 0.35 in. per day from a standard evaporation pan, it requires only a few days to develop severe drought conditions for all kinds of plants. As long as the soil is well supplied with moisture, the air itself is kept too humid by evaporation and transpiration to produce that amount of evaporation from the open pan. It is only after the soil has become dry at the surface that the atmosphere can attain conditions causing 0.35 in. of evaporation per day.

By the time this condition is reached, plants are practically limited to the water supply found in the soil mass actually occupied by their root systems. The plants and soils dry down together, but always with the greatest forces for water movement developed in connection with the leaves. In other words, the saturation deficit of the atmosphere is first transferred to the leaf, and is then propagated back through the plant body to the root system, and through the root system into the soil itself. As the soil becomes more and more depleted of water through root absorption and evaporation, the capillary adjustment on the surfaces of the mineral particles and soil colloids becomes slower and slower, until it is finally completely ineffective in the delivery of water to the plant. This point defines the wilting coefficient of the soil, as was pointed out many years ago (19). The plant, threatened by relentless evaporation and unyielding soil, does what it can to adjust itself to its diminishing water content. Lower leaves begin to die; or, as in the case of the common poplar, the leaves may be abscised in great numbers, even in early summer. In cases where the drought is not so severe as to cause death or leaf fall, growth is slowed down, the new leaves are not as well expanded, and because of this fact show a larger number of stomata per unit area. MAXIMOV (12) has clearly outlined the stomatal responses of plants to atmospheric conditions. In a brief paper YOCUM (30) has recently described the situation with reference to oak trees. Height of insertion of leaves upon the tree does not affect stomatal numbers in the case of mature trees which produce their entire crop of leaves at one time in early spring. Branches that continue to grow and leaf throughout the season, however, bear smaller upper leaves, with increased numbers of stomata per unit area because of the lesser expansion of the new leaves formed during the drier summer season.

In a former paper (23) we have discussed the development of colloidal imbibitional forces in the cell walls bordering the intercellular spaces of the leaf, and the conversion of imbibitional forces into osmotic action in the mesophyll cells of the leaf. The development of such forces through saturation deficits is a necessary prerequisite for the ascent of sap. The columns of water will not move upward until the forces are sufficient to lift them and overcome all frictional resistance to movement.

From our studies of eosin absorption by submerged shoots and twigs (23) in which the saturation deficit had been largely relieved by provision of a free water supply following submergence, we have concluded that sap ascent is caused by the physical and chemical effects of evaporation on the cell colloids and the forces generated thereby, and is not associated with an active cellular excretion of water at the expense of respiratory energy. Both DIXON (3) and BOSE (1) thought that there was evidence of such secretion, but we were unable to observe it in saturated tissues. It is believed that DIXON's results rested upon the use of unsaturated tissues, as his results

were readily confirmed by experiments conducted according to DIXON's description of his work. In a recent paper VAN DER PAAUW (13) has criticized our method, and claims that by the use of very sensitive potometers he has demonstrated a long continued intake of water by submerged cut shoots. He thinks eosin may be adsorbed at the lower end of the tracheae as fast as it enters when entry is slow, and that the method thus may fail to record sap rise even when it occurs. This criticism by VAN DER PAAUW is not taken very seriously, because our results contrast rather sharply with the eosin experiments of DIXON which would naturally be subject to the same criticism. Moreover, we found long continued intake in leafless submerged twigs with the eosin method even when entry was very slow. This slow entry of water over long periods was attributed in part to changes in gas volume in the tracheae, possibly slow solution of the gases. I am inclined to think that VAN DER PAAUW's results, which involve only small volumes of water, are also related to changes in gas volumes in the tracheae. VAN DER PAAUW does not find anything in his results to support the secretion hypothesis. He merely objects to our conclusion that water does not enter the shoots after they have been saturated. The decision in this case might hinge upon how one defines saturation. At any rate the facts as far as we know them favor the belief that sap ascent is caused by physical and chemical forces which are generated by evaporation of water from imbibed colloidal structures.

From the data presented in table I it can be seen that it does not require a large saturation deficit to accomplish the work of sap ascent. A leaf-colloid vapor pressure deficit of not over one per cent. will provide a stromogenic tension (7) sufficient to supply water to a height of 75 ft. This is estimated on a basis which allows as much force to overcome friction as to lift the columns of water. It also allows a liberal deduction for the net osmotic pressure of the root cells which must be overbalanced by the net osmotic pressure of the leaf cells. A 2 per cent. vapor pressure deficit would provide a tensional pull sufficient for all vegetation except the tallest trees; and a 3 per cent. deficit would provide stromogenic tension greater than that required for the tallest redwoods and eucalypts.

The saturation deficit of the leaf is not constant, but fluctuates rhythmically. It is low in the early morning as a rule, rises during the middle of the day, and falls again at night. This fluctuation of the imbibitional and osmotic values of leaf cells has been observed by many workers. It is caused by several factors working together. In the first place, the increasing transpiration decreases the volume of the cell, and, by concentrating the solutes of the cell sap, decreases the free water of the cell. At the same time, there is increased sugar manufacture in the mesophyll cells, which tends also to decrease the free water. In addition, JIMBO (8) claims that there is some conversion of starch or other condensation products to simpler soluble materials which helps to emphasize the saturation deficit during the middle of the day.



The movement of water from cell to cell in the leaf is controlled by differences in the net osmotic pressure. This term is used to designate the excess of osmotic pressure over wall pressure. It corresponds to URSPRUNG'S (27) suction force. For instance, if the osmotic pressure within the cell is 6 atmospheres, and the wall, through being stretched, is pressing upon the protoplasm with a force of 2 atmospheres, the net pressure of the cell is 4 atmospheres. If an adjoining cell happens to have a lower net pressure, water will move by osmosis (diffusion) from the cell of lower net pressure to the cell of higher net pressure. A gradient of net osmotic pressure extends across the mesophyll cells which lie between the intercellular spaces, where evaporation occurs, and the tracheal conducting system. As cells approach incipient plasmolysis, wall pressure becomes smaller and smaller, until the entire osmotic concentration of the cell expresses itself in the diffusional osmotic movement of water into the cell. The osmotic force of a leaf just at the point of incipient plasmolysis may in extreme cases even in normal life (6) reach values as high as 200 atmospheres. It is, however, usually much lower than this. In his studies of prairie plants STODDART (24) reports concentrations ranging from 20 to 60 atmospheres at the time the plants were dying from drought.

As water leaves the tracheae in response to these imbibitional and osmotic conditions of the leaf, a stromogenic tension is created in the water columns of the tracheal system. The cohesion of water is very great, and the evidence in favor of the cohesion theory of DIXON is fairly convincing. There are many details, however, that need clarification. The best interests of science will be served if, as has been suggested, it is used as a working hypothesis to be tested in every possible manner. Certainly this theory is better grounded than any of the substitutes that have been offered under the name of sap hydraulics. The inclusion of sorption of water on the tracheal walls as a part of the theory of sap rise does not aid us in the theoretical consideration of this problem, because the same force that sustains the columns or films of water so that they do not run out of the tracheae when the stems are cut, will also oppose the upward movement of the columns with exactly the same force. Moreover, the old tensile film theory of QUINCKE, which was discarded many years ago by DIXON as inadequate, but which has recently been revived by PEIRCE (14, 29) and his students, cannot be accepted until it is shown that the reasoning on which it was originally discarded is not sound. That has not been done. Finally, it is difficult to see how a central core of vapor in the tracheae could be of any possible advantage in sap ascent, because there seems to be no way in which traction can be obtained in such a column. While evaporation might occur at one end of the system and condensation at the other, the temperature differences favor evaporation above, and condensation at the lower ends of the tracheae, the reverse of what is demanded. As yet we have no real evidence of such vapor

transfer in sap ascent. Such proposals leave one feeling that they are only expressions of opinion, rather than theories dependent for their validity upon an array of supporting evidence. It seems clear, therefore, that we do not yet have any good substitute for the cohesion theory, nor any sound reasons for discarding it at the present time. It is true that we do not understand just what may be the importance of the pneumatic portions of the tracheal system. The reduced air pressures developed within the pneumatic tracts may contribute in some manner to the ascent of sap. Certainly the sudden chilling of the pneumatic system while it is under severe tension, causes large increases in the rate of water intake by cut branches as measured by potometer readings.

The water deficit which is first established in the leaves of the plant is transmitted to the root system through the action of the stromogenic tension in the cohesive water system of the tracheae. Under the stresses developed, water may simply pass from the living cells of the root into the tracheae because the stromogenic tension exceeds the net osmotic pressure of these cells. Such movement of water would establish a saturation deficit in the interior of the root, which would be extended toward the periphery of the root by a combination of osmotic and imbibitional changes. Just as, in the leaf, gradients exist between tracheae and evaporating cells, so gradients of net osmotic pressure are set up in the roots which lead to the movement of free water from the peripheral cells inward toward the tracheal vessels. As soon as the saturation deficit extends to the epidermal cells of the root, the colloidal walls of these cells begin to absorb water from the surfaces of the soil particles which are in contact with the root absorbing cells. The forces involved in root absorption have been considered in a previous paper (22).

There has been some disagreement as to the most active regions of root absorption. Some physiologists have considered the root hairs as the main absorbing organs, others have championed the idea that the young portions of the roots where elongation is most rapid and where hairs have not yet been developed are the most active regions of absorption. Root hairs are then considered to have merely, or at least mainly, an anchorage function. The classical view has been that the root hairs are active absorbers, and specifically adapted by form and chemical constitution for their function. There is hope that the truth may soon be established with reference to this difference of opinion, for ROSENE and LUND (16) have described a method by which the absorptive activity of any given region of the root may be measured. The results obtained with onion roots indicate that the most active regions of absorption are not just back of the tip, and not in the most rapidly elongating region, but in a region 40 to 50 mm. back of the apex of the root. They do not say whether these onion roots possessed root hairs. But if roots generally prove to be most active 4 to 5 cm. from the tip, the main absorption is in the region where hairs are well developed, and not yet old.

As long as the soil is well provided with water, the root system maintains a rate of water intake usually sufficient to prevent any serious consequences from transpirational water loss. But if the soil as well as the atmosphere becomes arid, the root system can absorb only the quantity of water that is delivered to its surface by capillary movement of water. When the soil particle surface films reach a certain degree of thinness, capillarity is no longer able to deliver a sufficient amount of water to the roots. Those soil particles which are in actual contact with the root hairs become much drier than other soil particles just beyond the limits of the root hairs. Careful observation shows that there is a sharp line of demarcation between the drier and moister particles, and that capillarity has ceased to function. At this point the plant must wilt, no matter how much change occurs in the imbibition pressures of cell walls and protoplasm, no matter how high osmotic values are produced in the vacuoles of the leaf cells, no matter how great stromogenic tension is developed in the tracheae; water cannot be obtained when it is not available, and permanent wilting and death soon follow. Good xerophytes may live for a long time without much water intake, but they do not grow. It is this ability to withstand severe water deficits without actually being killed that distinguishes the true xerophyte.

In one of my early papers (19) it was stated that at the wilting coefficient, the soil is withholding water from the plant by only about four atmospheres, and that the root cells have an osmotic pressure of about eight atmospheres if HANNIG'S (5) values are accepted as average. In other words there is a good gradient for water movement into the plant, but no significant intake at the wilting point. In this connection I called attention to work by ROBERTS (15) and TRUE (25) in which it had been found that plant cells adjust their internal concentrations so as to maintain a few atmospheres of diffusional gradient for water intake. MAXIMOV (12) has criticized my interpretation because of the fact that I made no distinction between osmotic concentration and the net pressure. So far as my own work is concerned this criticism is unjustified, however, for my measurements concern plants at the time of permanent wilting, at which time there could not possibly be any wall pressure to deduct from the osmotic pressure. At incipient plasmolysis, when wilting first occurs, the total osmotic pressure, equivalent to the osmotic concentration, is available for work. The criticism may apply, however, to the conclusions concerning the results of ROBERTS and of TRUE in which wall pressures were probably not taken into consideration.

There has been a tendency in recent years to minimize the importance of soil capillarity with reference to water movement in response to root absorption. One difficulty is that the studies of water movement are usually made on disturbed soils. By suitable tunnelling of soils it should be possible to study capillary movements in undisturbed soil masses. The physical structure of disturbed soils is always different from that of natural soils and

could hardly be expected to show normal behavior. This may account for some of the low measurements of capillary water movement obtained by soil scientists. As long as the soil contains moisture above the wilting coefficient one would expect capillary action, more rapid when near the field capacity, and less and less rapid as the soil dries toward the wilting coefficient. In the range of soil moisture between the wilting coefficient and air dry condition, capillarity disappears as a factor. Water movement would then be confined to diffusion of water vapor through the soil from moister to dryer regions, or from warmer to colder regions.

In the moisture range just slightly above and below the wilting coefficient, root extension and development of new root hairs may be important in tapping new water supplies in the soil. In a sense the plant may go after water when water ceases to move adequately toward the plant; but certainly growth of the roots could not continue very actively with the medium of growth close to the wilting coefficient and with the top of the plant suffering large saturation deficit.

### Summary

In conclusion one may summarize the relations as follows: the plant body is a mass of organic colloidal materials organized for the processes of living. These colloidal materials cannot function in life unless they are well supplied with water. In addition to colloids and water, there are inorganic ions and molecules, and organic molecules, in solution. The water in the plant is part of the general water system of nature which has a vapor phase in the atmosphere, and both liquid and vapor phases in plants and soils.

The plant body is, under normal conditions of water supply, in a much swollen condition. The colloidal materials are swollen by imbibition, and the organized cells are swollen by osmotic pressure. This swollen condition is subject to the balance between water absorption through one permeable region of the plant (the root), and water loss through another permeable region, the shoot. If loss is greater than gain, swelling decreases because of saturation deficit. If gain is greater than loss at any time during such a period of deficit, the swelling again increases. During the autokinetic phase of plant growth, absorption is on the average more rapid than water loss, because the increasing size of the plant body requires, in addition to the replacement of transpirational loss, more water to satisfy the needs of its newly created protoplasm, cell walls, and vacuoles. This increasing mass of water represents the retentive capacity of the materials for water. During the autostatic phases of the life cycle, water loss exceeds gain as the plant body ripens. This is particularly true of annual plants.

The shrinking of the plant body under evaporational water loss generates colloidal surface forces which are propagated through the plant body, from leaf to stem, from stem to root, from root to soil. The generation and propa-

gation of these forces tend to hasten water absorption as long as the soil possesses enough free water to permit the speeding up of absorption. If the soil too is becoming dry, this same generation of forces in the leaf continues, with more and more shrinkage of colloids and cell vacuoles, and generation of greater and greater forces. The soil keeps on supplying water at rates set by its physical and chemical properties, and by the forces generated on the surfaces of the soil particles themselves. If the process goes far enough, the plant first becomes less active, then ceases to grow, after which it continues to dry out until it and the soil have reached the stage represented by the permanent wilting of the plant and the wilting coefficient stage of soil moisture. Adequate precipitation at any earlier stage than permanent wilting would reverse these processes. The body would begin to swell, the wilted leaves to revive, the meristems to renew active growth, and the plant as a whole to resume normal living. Permanent wilting, if prolonged, usually means death.

The forces of sap ascent reside first of all in the water molecules themselves, which by evaporating from colloidal materials generate the forces for sap ascent. These forces are transformed from imbibitional to osmotic action in the leaves, and express themselves as stromogenic tensions in the hydrostatic and pneumatic vascular tracts of the plant. If not all of the details of sap ascent are clear, at least the main outlines of the cohesion theory seem to be well grounded.

These water relations are important because maximum plant production depends upon the plant's ability, up to the limit of its inherent or hereditary capacity, to gather in materials and create plant substances from them. A thorough understanding of all of the water relations is fundamental to an intelligent management of the plant's environment. The function of this paper is to clarify particularly the relation of the water vapor phase of the atmosphere to the water economy of plants in general.

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# STUDIES ON ANTAGONISTIC PHENOMENA AND CATION ABSORPTION IN TOBACCO IN THE PRESENCE AND ABSENCE OF MANGANESE AND BORON

T. R. SWANBACK

(WITH TEN FIGURES)

## Introduction

Tobacco is very sensitive to slight variations in cultural or nutritional conditions, and is thus particularly suitable for physiological studies. Most tobacco is ultimately to be burned (smoked), and as a result great interest centers in the burning qualities of the leaf and the relation of these to the ash resulting and to factors governing ash composition. All of the above items in turn are reflected in the growth of the plant. The object of the present investigation was to study, by means of water cultures, the absorption of mineral nutrients. The bases, and factors governing their translocation within the plant, were studied specifically.

## Methods

In beginning experiments, a cultural method described by LUNDEGÅRDH (6) was employed. Tobacco plants of Havana seed type about four weeks old, were grown in sterile quartz sand with added weak nutrient solution. These were transferred to glass tubes about 45 mm. in diameter, of 200 to 250 ml. capacity, and filled with a measured quantity of a nutrient solution. The plants, one for each vessel, were fastened in a cork stopper 40 mm. in diameter,

TABLE I

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES.  
CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	A (LOW K CONTENT)						
		CONCENTRATION						
		N	P	K	CA	Mg	MN	B
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O .....	0.847	0.100			0.143			
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O .....	0.156		0.017					
Variable								
A B C								
K <sub>2</sub> SO <sub>4</sub> .06; .185; .6 .....				0.026				
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.308					0.030		
MnCl <sub>2</sub> · 4H <sub>2</sub> O .....	0.001						0.0003	
H <sub>3</sub> BO <sub>3</sub> .....	0.005							0.0008
Fe-citrate .....	0.01							
Total .....		0.100	0.017	0.026	0.143	0.030	0.0003	0.0008



TABLE I—(Continued)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES.  
CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	B (MEDIUM K CONTENT)						
		CONCENTRATION						
		N	P	K	Ca	Mg	Mn	B
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O .....	0.847	0.100			0.143			
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O .....	0.156		0.017					
Variable								
A B C								
K <sub>2</sub> SO <sub>4</sub> .06; .185; .6 .....				0.082				
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.308					0.030		
MnCl <sub>2</sub> · 4H <sub>2</sub> O .....	0.001						0.0003	
H <sub>3</sub> BO <sub>3</sub> .....	0.005							0.0008
Fe-citrate .....	0.01							
Total .....		0.100	0.017	0.082	0.143	0.030	0.0003	0.0008

TABLE I—(Concluded)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE POTASSIUM SERIES.  
CONCENTRATIONS GIVEN IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	C (HIGH K CONTENT)						
		CONCENTRATION						
		N	P	K	Ca	Mg	Mn	B
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O .....	0.847	0.100			0.143			
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O .....	0.156		0.017					
Variable								
A B C								
K <sub>2</sub> SO <sub>4</sub> .06; .185; .6 .....				0.26				
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.308					0.030		
MnCl <sub>2</sub> · 4H <sub>2</sub> O .....	0.001						0.0003	
H <sub>3</sub> BO <sub>3</sub> .....	0.005							0.0008
Fe-citrate .....	0.01							
Total .....		0.100	0.017	0.26	0.143	0.030	0.0003	0.0008

which was supported by a wire folded over the edge of the tube. Blackened cardboard folded around the tubes excluded light from the roots. The tubes, in triplicate for each treatment, were placed in a rack and transferred to a photothermostat (6). The nutrient solutions are given in tables I and II and the cultural variations in tables III, IV, and VII.

In all of the solutions purest Kahlbaum salts were used. In order to obtain a true, clear solution where it was necessary to employ calcium sulphate, a saturated solution of this salt was used (solubility in water of room temperature about 0.23 per cent.). The solution was analyzed as to Ca content and a quantity was added sufficient to meet the Ca requirements of the different

nutrient solutions. The reaction of solutions was equalized as nearly as possible at the beginning of the experiment. It varied slightly from a pH of 5.2 to 5.4. The solutions were changed weekly and plants were harvested after 45 days.

In later experiments, seedlings were grown in a soil flat. After six weeks they were transferred to one-liter Jena glass beakers each containing a measured quantity of nutrient solution. The beakers had been treated for about a month with a weak HCl solution. They were then thoroughly rinsed

TABLE II

COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES.  
CONCENTRATIONS IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	A (LOW Ca CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	0.25	0.03			0.042			
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ .....	0.156		0.017					
$\text{KNO}_3$ .....	0.214	0.03		0.082				
$\text{NaNO}_3$ .....	0.037	0.006						
$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ .....	0.32	0.034				0.03		
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....								
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.001						0.0003	
$\text{H}_3\text{BO}_3$ .....	0.005							0.0008
$\text{K}_2\text{SO}_4$ .....								
Fe-citrate .....	0.01							
$\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ .....	0.32							
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .....								
Total .....		0.100	0.017	0.082	0.042	0.03	0.0003	0.0008

TABLE II (Continued)

COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES.  
CONCENTRATIONS IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	B (MEDIUM Ca CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ .....	0.847	0.100			0.143			
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ .....	0.156		0.017					
$\text{KNO}_3$ .....								
$\text{NaNO}_3$ .....								
$\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ .....								
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ .....	0.308					0.03		
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ .....	0.001						0.0003	
$\text{H}_3\text{BO}_3$ .....	0.005							0.0008
$\text{K}_2\text{SO}_4$ .....	0.185			0.082				
Fe-citrate .....	0.01							
$\text{Na}_2\text{SO}_4 \cdot 10\text{H}_2\text{O}$ .....								
$\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ .....								
Total .....		0.100	0.017	0.082	0.143	0.03	0.0003	0.0008

TABLE II (*Concluded*)COMPOSITION OF NUTRIENT SOLUTIONS IN THE CALCIUM SERIES.  
CONCENTRATIONS IN GRAMS PER LITER

SALTS	CON- CENTRA- TION	C (HIGH CA CONTENT)						
		N	P	K	Ca	Mg	Mn	B
	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>
Ca(NO <sub>3</sub> ) <sub>2</sub> · 4H <sub>2</sub> O .....	0.847	0.100			0.143			
NaH <sub>2</sub> PO <sub>4</sub> · H <sub>2</sub> O .....	0.156		0.017					
KNO <sub>3</sub> .....								
NaNO <sub>3</sub> .....								
Mg(NO <sub>3</sub> ) <sub>2</sub> · 6H <sub>2</sub> O .....								
MgSO <sub>4</sub> · 7H <sub>2</sub> O .....	0.308					0.03		
MnCl <sub>2</sub> · 4H <sub>2</sub> O .....	0.001						0.0003	
H <sub>3</sub> BO <sub>3</sub> .....	0.005							0.0008
K <sub>2</sub> SO <sub>4</sub> .....	0.185			0.082				
Fe-citrate .....	0.01							
Na <sub>2</sub> SO <sub>4</sub> · 10H <sub>2</sub> O .....								
CaSO <sub>4</sub> · 2H <sub>2</sub> O .....	1.13				0.26			
Total .....		0.100	0.017	0.082	0.403	0.03	0.0003	0.0008

with distilled water to eliminate, as far as possible, contamination from the glass. The beakers were covered with blackened cardboard and on top of each was placed a sheet of black-varnished galvanized iron, in the center of which was a hole fitted with a 5-cm. cork stopper for holding the plant. Before transfer from the soil flat, each plant was thoroughly rinsed in distilled water and placed in a beaker. Non-absorptive cotton was packed around the stem of each plant. Each nutrient variant was set up in quadruplicate. The beakers were placed in artificially illuminated photothermostatically controlled chambers, described by LUNDEGÅRDH (6). The plants were allowed to grow for 60 days. Nutrient solutions were changed in the middle of the growth period. At this time a slight precipitation was evident in high-calcium solutions.

Nitrate nitrogen was determined in the solutions by the disulphonic acid method, and phosphorus by the phosphoceruleomolybdic acid method. Solutions, salts, and plant materials were analyzed for cations by the quantitative spectrographical methods described by LUNDEGÅRDH (6).

### Experimental results

#### THE EFFECT OF VARYING QUANTITIES OF CA ON GROWTH AND ON THE ABSORPTION OF POTASSIUM, CALCIUM, AND MAGNESIUM

Data for plant growth and cation absorption are given in tables III and IV; the first represents results from an experiment set up under the first procedure outlined under "Methods"; the second, under the revised procedure.

The data in table III show a marked response in growth as measured by

TABLE III

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS  
WITH VARYING AMOUNTS OF CALCIUM,\* EXPERIMENT 1

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
	PLANT	RELA- TIVE	K		Ca		Mg	
			PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	<i>gm.</i>		<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>
Low Ca								
Stems, leaves .....	0.416	46	1.240	0.516	0.259	0.108	0.330	0.137
Roots .....	0.110		1.030	0.113	0.052	0.016	0.285	0.031
Total plant .....	0.526			0.629		0.124		0.168
Medium Ca								
Stems, leaves .....	0.845	100	0.810	0.684	0.472	0.399	0.135	0.114
Roots .....	0.300		0.690	0.207	0.085	0.026	0.180	0.054
Total plant .....	1.145			0.891		0.425		0.168
High Ca								
Stems, leaves .....	1.010	120	0.760	0.768	0.426	0.430	0.086	0.087
Roots .....	0.370		0.720	0.266	0.089	0.033	0.202	0.075
Total plant .....	1.380			1.034		0.463		0.162

\* Low Ca = 1.05 millimols per liter.

Medium Ca = 3.50 " " "

High Ca = 10.2 " " "

TABLE IV

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS  
WITH VARYING AMOUNTS OF CALCIUM,\* EXPERIMENT 1

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
	PLANT	RELA- TIVE	K		Ca		Mg	
			PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	<i>gm.</i>		<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>	<i>m. mol</i>
Low Ca								
Leaves .....	1.26	40	1.47	1.85	0.68	0.86	0.55	0.69
Stems .....	0.31		0.82	0.43	0.12	0.62	0.08	0.04
Roots .....	0.21							
Total plant .....	1.87			2.28		1.48		0.73
Medium Ca								
Leaves .....	2.47	100	1.73	4.27	1.06	2.62	0.27	0.67
Stems .....	1.35		1.34	1.81	0.12	1.62	0.08	0.11
Roots .....	0.45		0.68	0.31	0.21	0.10	0.20	0.09
Total plant .....	4.27			6.39		4.34		0.87
High Ca								
Leaves .....	7.28	360	1.27	9.25	0.93	6.77	0.20	1.46
Stems .....	6.39		0.59	3.77	0.14	0.89	0.02	0.13
Roots .....	1.71		0.38	0.65	0.14	0.24	0.20	0.34
Total plant .....	15.38			13.67		7.90		1.93

\* Low Ca = 1.05 millimols per liter.

Medium Ca = 3.50 " " "

High Ca = 10.2 " " "

dry weight with increment of Ca in the nutrient solution. Dry matter production with the medium supply is more than double that of the low supply, although the high supply produced only 20 per cent. more dry matter than the medium. Stems and leaves increased in about the same proportion as the roots. In figure 1 a graphical presentation of the relation between growth, Ca supply, and Ca absorption is given.

Of greater interest, however, is the absorption of certain elements as reflected by the ash analyses. Absorbed Ca increased 45.3 per cent. per unit of dry matter above that for "low Ca" with the addition of about three times as much Ca as in the low solution. Further addition of calcium, however, lowered the Ca content per unit dry matter in the stems and leaves, although the total per plant increased but not in proportion to added Ca.

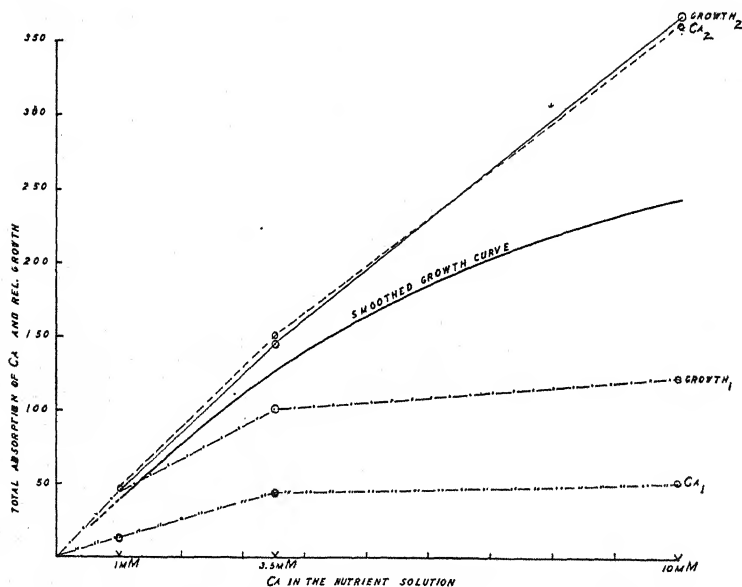


FIG. 1. Graphs showing the relation of growth to calcium supplied in the nutrient solution. The smoothed growth curve shows the general trend of experiments 1 and 2.

Considering the percentage content of Ca in the stems and leaves only, with "medium" as 100, the relationship of the low, medium, and high cultures was about 55, 100, and 90 respectively. The corresponding figures for K were about 153, 100, and 94 respectively, and for Mg, 244, 100, and 64. A low Ca supply in the nutrient medium causes a variation in the absorption of the three bases which occur in the plant in the order  $Mg > K > Ca$ , while at a high Ca supply the variation is slight and the order of occurrence is  $K > Ca > Mg$ . Since the order of Mg is directly reversed and the only

variable to account for this change is Ca, the antagonism Ca:Mg is plainly established.

A slight K:Ca antagonism is suggested which is emphasized, however, in following experiments where K is the variable. No Ca:K antagonism is indicated in figure 2 where the K content shows a considerable drop at medium Ca supply. With low Ca supply (1 millimol), and with K at 2.1 millimols, any antagonism that Ca may have possessed for K was entirely obscured by the differences in concentration of the two elements.

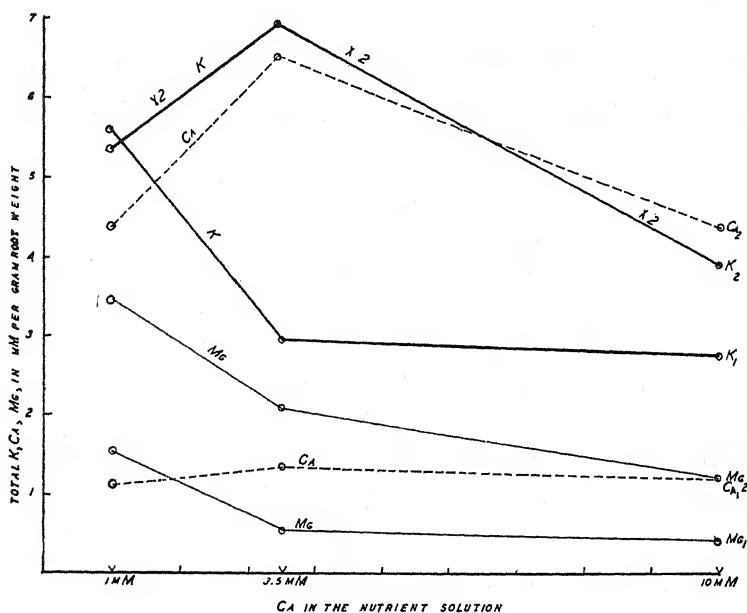


FIG. 2. Graphs showing the relation of total potassium, calcium, and magnesium absorbed per gram of root weight to varying supply of calcium in the nutrient solutions (experiments 1 and 2).

In the second experiment, table IV, with a growing period of 60 instead of 45 days, the high Ca supply produced a considerably higher yield of dry matter than shown in table III. The resulting growth was approximately proportional to Ca supplied in the nutrient solution as evident from relative weights and from figure 1. The data show that stem production was most influenced by Ca, followed by roots, with leaves least influenced by this element. These data also show that Ca noticeably affected the absorption of Mg, with no antagonistic effect on K. A slight pseudo-antagonism Ca:K is suggested at the highest Ca level, more plainly shown in figure 2.

The interrelationship of the three bases, K, Ca, and Mg, is similar to that in the first experiment; for K the relative figures for a low, medium, and

high Ca supply is 78, 100, and 60; for Ca, 64, 100, and 57; and for Mg, 275, 100, and 44 respectively. The order for the three bases at the low Ca supply is  $Mg > K > Ca$ , and at the high Ca supply,  $K > Ca > Mg$ , which is identical with the results noted above.

The total absorption of the three bases at a low, medium, and high Ca supply, expressed by ratios with low Ca as 1, was: K, 1, 2.8, and 6.0; Ca, 1, 2.9, and 5.3; and Mg, 1, 1.2, and 2.6. With one exception, the absorption of the three bases progressively increased with increased Ca supply and, incidentally, dry matter production increased. The growth curve shown in figure 1 runs almost parallel to the curve of total Ca absorption. A smoothed growth curve shows the general trend of response to Ca as a growth factor.

THE EFFECT OF VARYING QUANTITIES OF POTASSIUM ON GROWTH AND ON  
ABSORPTION OF POTASSIUM, CALCIUM, AND MAGNESIUM

In the second series of experiments K was varied and the resulting data are given in tables V and VI. A set of cultures containing no K was also included; these plants lived through the growing period but showed no progress in growth. In a trial without Ca, the plants soon died. In this comparison it is evident that the plants grown without K were able through transfer of K to progressively younger parts to maintain life, while the

TABLE V

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS  
WITH VARYING AMOUNTS OF POTASSIUM,\* EXPERIMENT 1

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
			K		Ca		Mg	
	PLANT	RELA- TIVE	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
	<i>gm.</i>		<i>m.mol</i>	<i>m.mol</i>	<i>m.mol</i>	<i>m.mol</i>	<i>m.mol</i>	<i>m.mol</i>
No K .....	0.060	5.2	Trace		0.770		0.390	
Low K								
Stems, leaves ...	0.720		0.480	0.346	0.644	0.464	0.152	0.109
Roots .....	0.280		0.460	0.129	0.069	0.193	0.240	0.067
Total plant .....	1.000	87.3		0.475		0.657		0.176
Medium K								
Stems, leaves ...	0.845		0.810	0.684	0.472	0.399	0.135	0.114
Roots .....	0.300		0.690	0.207	0.085	0.026	0.180	0.054
Total plant .....	1.145	100.0		0.891		0.425		0.168
High K								
Stems, leaves ...	0.940		1.530	1.438	0.086	0.081	0.088	0.083
Roots .....	0.250		1.250	0.313	0.090	0.023	0.103	0.025
Total plant .....	1.190	104.0		1.751		0.104		0.108

\* Low K=0.66 millimols per liter.

Medium K=2.10   "   "   "

High K=6.66   "   "   "

no-Ca plants succumbed from failure to replenish the Ca. As seen in table V, only a trace of K was found in the plant material when this element was lacking in the solution, while Ca and Mg contents were the highest in this group of cultures.

With varying amounts of K in the nutrient solution (table V and fig. 3) the production of dry matter varies much less than in the case of Ca. From data in table V it is calculated that K increased 40.7 per cent. per unit of dry

TABLE VI

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF POTASSIUM,\* EXPERIMENT 2

TREATMENT AND PLANT PARTS	DRY WEIGHT		ASH ANALYSIS					
	PLANT	RELA- TIVE	K		Ca		Mg	
			PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART	PER GRAM	PER PLANT PART
Low K	gm.		m.mol	m.mol	m.mol	m.mol	m.mol	m.mol
Leaves .....	8.70		0.50	4.35	1.39	12.09	0.39	3.39
Stems .....	6.65		0.26	1.73	0.24	1.60	0.13	0.86
Roots .....	1.97		0.13	0.26	0.11	0.22	0.44	0.87
Total plant .....	17.31	320		6.34		13.91		5.12
Medium K								
Leaves .....	2.47		1.73	4.27	1.06	2.62	0.27	0.67
Stems .....	1.35		1.34	1.81	0.12	1.62	0.08	0.11
Roots .....	0.45		0.68	0.31	0.21	0.10	0.20	0.09
Total plant .....	4.27	100		6.39		4.34		0.87
High K								
Leaves .....	4.89		2.10	10.27	0.80	3.91	0.33	1.61
Stems .....	3.59		1.38	04.95	0.09	0.32	0.05	0.18
Roots .....	0.93		1.28	01.19	0.17	0.16	0.17	0.16
Total plant .....	9.41	220		16.41		4.39		1.95

\* Low K=0.66 millimols per liter.

Medium K=2.10 " " "

High K=6.66 " " "

matter when about three times as much K was added for "medium K" as for "low K." Although further additions of K increased K per unit of dry weight, this latter did not respond to further additions, notwithstanding excessive absorption of this element. The relative content of the three bases in stems and leaves at a low, medium, and high K supply was for K: 60, 100, and 190; for Ca: 140, 100, and 18; and for Mg: 113, 100, and 65. Thus, at the low K supply the order in which the bases were absorbed was: Ca > Mg > K; and for the high K supply, K > Mg > Ca. Since Mg kept its place in the rank and since K was the only variable, the antagonism K: Ca is suggested.

There is an apparent pseudo-antagonism, Ca: K at the low K supply (0.66



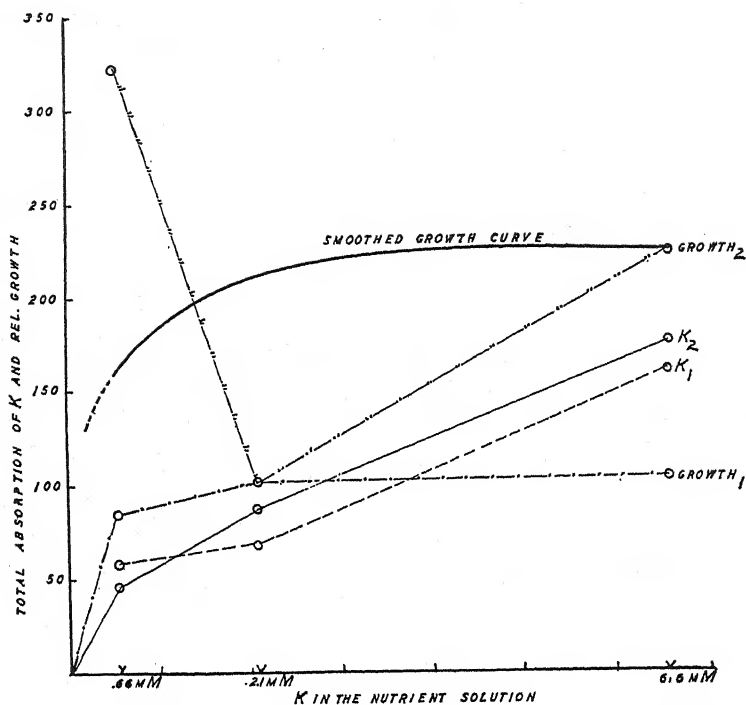


FIG. 3. Graphs showing the relation of total potassium to growth affected by varying the potassium in the nutrient solutions.

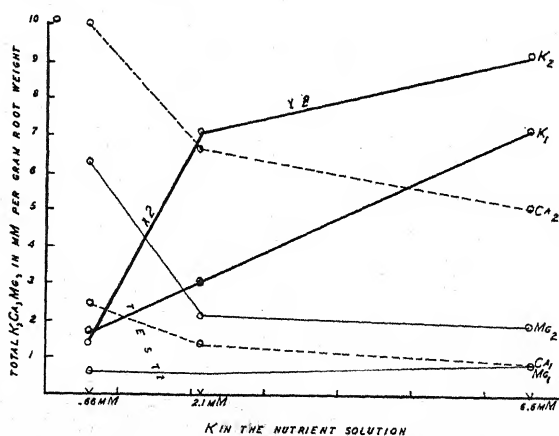


FIG. 4. Graphs showing the relation of total potassium, calcium, and magnesium absorbed per gram of root weight to varying supply of potassium in the nutrient solutions.

millimols per liter), while Ca was present at a concentration of 3.5 (fig. 4). Obviously, a true antagonism did not occur if this instance is compared with the one of medium K supply (2.1 millimols), with Ca constant as above. This K concentration caused a considerable drop in the Ca absorption. Therefore, Ca cannot be considered antagonistic to K although pseudo-antagonism is suggested when the concentration of Ca is vastly higher than that of K.

In the revised experimental procedure, the results of which are given in table VI, K had no direct influence on dry matter production at the concentrations used. In fact, the highest relative weight was produced with the lowest addition of K. It appears that a somewhat lower concentration of K would have been sufficient to produce normal growth.

With the increasing K supply in the nutrient solution, K progressively increased and Ca decreased, while Mg was only slightly affected. The inter-relationship of the bases as previously computed is for K: 24, 100, and 109; for Ca: 154, 100, and 69; and for Mg: 130, 100, and 95. At a low K supply the order of base absorption is  $\text{Ca} > \text{Mg} > \text{K}$ ; at a high K supply,  $\text{K} > \text{Mg} > \text{Ca}$ . This ranking coincides with results of the previous experiment.

#### EFFECT OF MANGANESE ON GROWTH AND ON THE ABSORPTION OF POTASSIUM AND CALCIUM

In the absence of Mn in the nutrient solution, deficiency symptoms of this element were pronounced at "high Ca," less at medium, and not noticeable at a "low Ca" supply. The symptoms first occur on the younger leaves (not

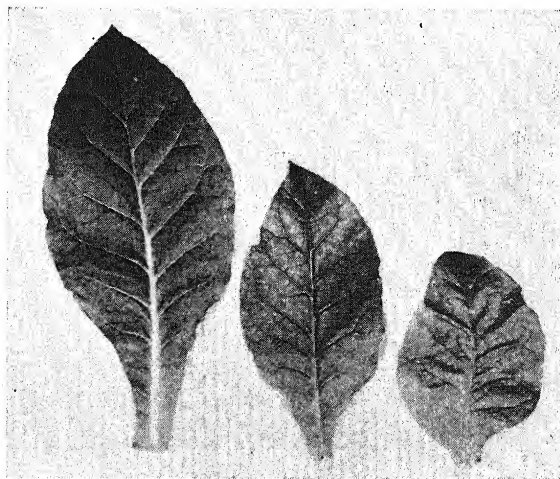


FIG. 5. Leaves from tobacco plants grown (to the left) with manganese and (the two to the right) without manganese.

the youngest) in the form of a chlorosis between the veins and resembles Mn toxicity (6) which, however, appears first at the growing point. The checkered appearance is not as firmly manifested as in the case of the toxicity. In some cases the leaves were crinkled and the size reduced (fig. 5). The roots at "high Ca" without Mn were like those described by McMURTREY (9), *i.e.*, "They did not show many branches but those they had were relatively long." The roots of these cultures were the only ones to reach the bottom of the 1000-ml. containers.

Symptoms of Mn deficiency were very pronounced in "low K" plants, less in "medium," and not noticeable in "high K" plants. The effects with K were thus in reverse order from those with Ca which suggests that Ca is antagonistic to Mn, while K is indifferent.

The data given in table VII show that the presence and absence of Mn (0.0054 millimol per liter) produced vast differences in dry matter in certain instances. With a low Ca supply dry matter in the absence of Mn was six times that obtained where Mn was present. At high Ca supply conditions were reversed so that about 3.5 times as much dry matter was produced in the presence of Mn as in its absence. These results suggest that at a low Ca supply without Mn, there being no antagonism of Mn toward Ca, the Ca could be utilized to the limit. At the high Ca supply the antagonistic action of Mn on Ca was not sufficient to prevent absorption of Ca.

The data of table VII show similar results for "high Ca" and "low K." The latter without Mn resembles "high Ca" with Mn. The presence or absence of Mn at high supply had little influence on growth, suggesting that the antagonism K:Ca obscured the effects of the absence of the essential Mn. The slightly larger weight where Mn was absent might suggest that an antagonism Mn:K was responsible.

The result of these experiments on the absorption of bases in the absence of Mn cannot be considered conclusive since the normal functions of the plant were obviously disturbed. The data of table VIII suggest, however, that Ca is antagonistic to Mn and that Mn is antagonistic to K.

The antagonistic phenomena in relation to Mn are more clearly shown from the standpoint of Mn absorption and translocation by varying the supply of Ca and K in the nutrient solution. Data from such an experiment are given in table VIII. The mobility of Mn, or its relative translocation from the roots to other parts of the plant, may be expressed by a ratio of the Mn content found in stems<sup>1</sup> and leaves and that found in roots. This

<sup>1</sup> No Mn was detected in the stems, when analyzed separately, irrespective of presence of Mn in the nutrient solution. This may be explained on the basis that there would not at any one time be a measurable amount present in the stem where Mn apparently is not stored. It suggests, moreover, that Mn is not a part of compounds formed in the leaf and later translocated into the stems. This is in line with the viewpoint that Mn acts as a catalytic agent.

TABLE VII

DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING QUANTITIES OF POTASSIUM AND CALCIUM; AND WITH AND WITHOUT MANGANESE\*

TREATMENT AND PLANT PARTS	DRY WEIGHT				ASH ANALYSIS							
	PLANT		RELATIVE		CONCENTRATION PER GRAM							
	MN		MN		K		CA		Mg			
	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT
Low Ca	gm.	gm.			m.mol	m.mol	m.mol	m.mol	m.mol	m.mol		
Lower leaves	1.26	5.55			0.98	0.99	0.72	0.99	0.55	0.65		
Top	0.31	3.33			1.19	0.36		0.36		0.51		
Stems	0.21	1.42			1.11	0.06		0.06		0.09		
Roots	1.78	10.30	40	240	0.49	0.08	0.14	0.08	0.08	0.31		
Total plant					2.28	4.20	1.48	4.20	0.73	3.96		
High Ca												
Lower leaves	7.28	2.85			1.57	1.54	1.54	1.71	0.19	0.34		
Medium "					1.10	0.88	0.88	0.23	0.23			
Top					1.15	1.54	0.38	0.66	0.18	0.30		
Stems	6.39	2.75			0.59	1.95	0.14	0.39	0.02	0.10		
Roots	1.71	0.79			0.38	0.43	0.13	0.19	0.20	0.54		
Total plant	15.38	6.39	368	150	13.67	11.20	7.90	4.67	1.93	1.59		
Low K												
Lower leaves	8.70	3.45			0.37	0.63	1.77	1.87	0.44	0.51		
Top	6.65	2.04			0.53	1.25	0.41	0.61	0.19	0.41		
Stems	1.97	0.57			0.26	0.65	0.24	0.29	0.13	0.21		
Roots	17.31	6.06	405	142	0.13	0.17	0.11	0.30	0.44	0.72		
Total plant					6.34	4.67	13.91	5.05	5.12	2.42		
High K												
Lower leaves	4.89	6.53			2.20	2.95	1.22	0.96	0.28	0.19		
Medium "					2.25	3.11	0.51	1.52	0.26	0.36		
Top					1.75	2.27	0.28	0.51	0.36	0.23		
Stems	3.59	2.45			1.38	2.03	0.09	0.29	0.05	0.10		
Roots	0.93	0.72			1.28	1.84	0.17	0.22	0.17	0.18		
Total plant	9.41	9.70	220	227	16.41	24.38	4.39	7.43	1.95	2.08		

\* Mn concentration 0.0054 millimol per liter.

TABLE VIII

MANGANESE CONTENT IN MILLIMOLS PER GRAM DRY WEIGHT OF TOBACCO PLANTS GROWN IN NUTRIENT SOLUTIONS WITH VARYING AMOUNTS OF CALCIUM AND POTASSIUM; AND TRANSLOCATION QUOTIENTS FOR MANGANESE

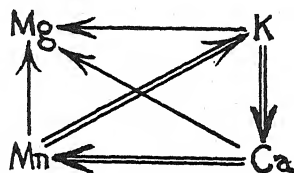
TREATMENT AND PLANT PARTS	GROWTH PERIODS			
	MANGANESE X 0.001 PER GRAM		MANGANESE TRANSLOCATION QUOTIENT	
	45 DAYS	60 DAYS	45 DAYS	60 DAYS
Low Ca	<i>m.mol</i>	<i>m.mol</i>		
Leaves, stems .....	0.74	0.75	0.91	1.25
Roots .....	0.82	0.60		
Medium Ca				
Leaves, stems .....	0.25	0.36	0.70	0.07
Roots .....	0.36	5.00		
High Ca				
Leaves, stems .....	0.16	0.33	0.61	0.06
Roots .....	0.26	5.60		
Low K				
Leaves, stems .....	0.22	0.20	0.43	0.10
Roots .....	0.51	2.10		
Medium K				
Leaves, stems .....	0.25	0.36	0.70	0.07
Roots .....	0.36	5.00		
High K				
Leaves, stems .....	0.30	0.28	0.61	0.09
Roots .....	0.49	3.20		

ratio is designated the translocation quotient (Tq). Data from translocation quotients in table VIII show that the least resistance to Mn absorption is offered by "low Ca" and the highest by "high Ca." Since "medium Ca" is intermediate a rather definite antagonism Ca:Mn is suggested.

With the low, medium, and high K supply there is no regularity in Mn translocation quotient relations. At the "low K" level, however, the translocation quotient even lower than that at "high Ca" suggests that increased Ca absorption incident to "low K" is responsible for reduced Mn translocation. In this connection it should be recalled that the relative absorption of Ca was greater at "low K" than at "high Ca."

Although no special Mg test similar to those with Ca and K was undertaken, the analytical data in table VI and VII indicate that the absorption of this element is depressed under low Ca and low K supplies in the presence of Mn. It appears, however, that the antagonism Ca:Mn is stronger than that of Ca:Mg. Therefore, it may be concluded that the rank of absorption may be Mn > Mg; K > Ca.

Graphically this may be visualized in the following way:



The arrows point in the direction of the antagonism, the heavy lines suggesting a strong antagonism, the lighter lines a weaker antagonism. Potassium is thus antagonized only by Mn which is in line with the excessive absorption of this latter element, when other elements do not provide a counter antagonism. According to this concept Mg is antagonized by all three of the other elements. Its absorption must be controlled and is possible only because of the counter antagonistic effects of the other elements on one another.

#### THE EFFECT OF BORON ON GROWTH AND ON THE ABSORPTION OF CALCIUM AND POTASSIUM

In a previous paper (17) the writer has shown that boron is essential for normal growth of tobacco plants. In the present work these findings have been confirmed. Deficiency symptoms (arrested top growth, crinkled and brittle leaves) occurred (fig. 6) soon after the experiment was begun



FIG. 6. Boron deficiency ("high Ca").

at low-Ca and -K and high-K supplies. At a high Ca supply the symptoms did not appear until the middle of the growing period. The reason for this may be that minute traces of B (too small to detect by any known analytical method) adhered to the Ca salt used to obtain the high Ca supply. Boron content was apparently sufficient to sustain growth for a certain length of time.

TABLE IX  
 DRY WEIGHTS AND ASH ANALYSES OF TOBACCO PLANT GROWN IN NUTRIENT SOLUTIONS WITH VARYING  
 QUANTITIES OF POTASSIUM AND CALCIUM; AND WITH AND WITHOUT BORON

TREATMENT AND PLANT PARTS	DRY WEIGHT				ASH ANALYSIS							
	PLANT		RELATIVE		CONCENTRATION PER GRAM							
	B		B		K				Ca			
					B				B		Mg	
	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT	WITH	WITHOUT
Low K	gm.	gm.			m. mol	m. mol	m. mol	m. mol	m. mol	m. mol	m. mol	m. mol
Lower leaves	1.26	0.71			1.47	1.23	0.72	0.68	0.55	0.78		
Top												
Stems	0.31	0.23			0.82	0.50	0.14	0.12	0.08	0.14		
Roots	0.21											
Total plant	1.78	0.94	40	20	2.28	0.99	1.48	0.51	0.73	0.58		
High K												
Lower leaves	7.28	3.31			1.57	1.30	1.54	1.35	0.19	0.17		
Medium					1.10		0.88		0.23			
Top		1.95			1.15	1.69	0.38	0.91	0.18	0.23		
Stems	6.39	1.35			0.59	1.13	0.14	0.52	0.02	0.07		
Roots	1.71	0.77			0.38	0.74	0.13	0.29	0.03	0.03		
Total plant	15.38	7.38	368	170	13.67	9.70	7.90	7.15	1.93	1.12		
Low K												
Lower leaves	8.70	0.84			0.37	1.05	1.77	1.05	0.44	0.47		
Top					0.53		0.41		0.19			
Stems	6.65	0.20			0.26	0.38	0.24	0.42	0.13	0.23		
Roots	1.97				0.13		0.11		0.44			
Total plant	17.31	1.04	405	20	6.34	0.96	13.91	0.97	5.12	0.44		
High K												
Lower leaves	4.89	1.94			2.20	2.22	1.22	1.49	0.28	0.36		
Medium					2.25		0.51		0.26			
Top		0.62			1.75	1.77	0.28	0.37	0.36			
Stems	3.59	0.84			1.38	1.77	0.09	0.15	0.05	0.09		
Roots	0.93	0.25			1.28	0.53	0.17	0.22	0.17	0.44		
Total plant	9.41	3.65	220	80	16.41	6.92	4.39	3.31	1.95	1.11		

Data from the boron experiment are found in table IX. In studying the ash analyses it is found that the presence or absence of B in the nutrient solution had a consistent effect on the absorption of the bases; in some instances, the plants without B accumulating more bases per unit of dry weight than those with B supplied. When this occurred it apparently was caused by retarded growth since normal plants were not obtained in the absence of B.

From the difference between the amounts applied and the amounts found at renewal of solutions and at the termination of the experiment, the amount of nitrate nitrogen absorbed by the plants was estimated. The absorption relationship only between treatments that showed great differences are considered. The data are given in table X. Invariably, more nitrates were

TABLE X

NITRATE NITROGEN ABSORBED IN MILLIMOLS PER GRAM DRY MATTER

TREATMENT		NITRATE-NITROGEN
		<i>m. mol.</i>
Low	Ca with B .....	12.8
"	Ca without B .....	25.8
High	Ca with B .....	3.3
"	Ca without B .....	7.6
Low	K with B .....	3.9
"	K without B .....	21.4
High	K with B .....	4.0
"	K without B .....	11.0

absorbed in the absence of B. The smallest difference appears in "high Ca." This is reflected in the small differences in cation absorption of the high-Ca treatments with and without B (table IX).

#### THE EFFECT OF VARYING QUANTITIES OF POTASSIUM AND CALCIUM ON THE ABSORPTION OF IRON, SODIUM, AND PHOSPHORUS

From the data in table XI it is suggested that the absorption of Fe is retarded by Ca. The approximate "Tq" for Fe is 1.0 at "low Ca," where in the cultures with larger amounts of Ca, the quotient is lowered to 0.20 or less. The accumulation of Fe in the roots in the presence of larger quantities of Ca might be due to a diminution of the pores in the cell walls which Ca is known to produce. It is possible also that insoluble Fe-compounds are formed and, therefore, not translocated away from the roots.

The absorption of Na was retarded more by Ca than by K. The "Tq" of Na would probably decrease rapidly with increasing supply of Ca, and increase with increasing K. It might be gathered that there is a stronger antagonism Ca:Na than K:Na.

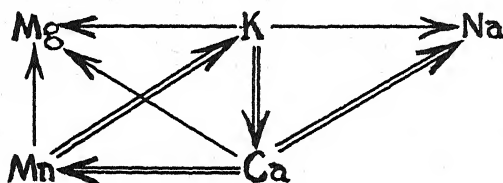


TABLE XI

THE CONTENT OF IRON, SODIUM, AND PHOSPHORUS IN MILLIMOLS PER GRAM DRY WEIGHT OF PLANTS GROWN WITH VARYING QUANTITIES OF CALCIUM AND POTASSIUM

TREATMENT	FE	T <sub>Q</sub> FE	NA	T <sub>Q</sub> NA	P	T <sub>Q</sub> P
	<i>m.mol</i>		<i>m.mol</i>		<i>m.mol</i>	
Low Ca						
Stems, leaves .....	0.005	1.0	0.29	0.42	0.14	0.30
Roots .....	0.005		0.69		0.46	
Medium Ca						
Stems, leaves .....	0.005	0.20	0.05	0.45	0.14	0.87
Roots .....	0.023		0.11		0.16	
High Ca						
Stems, leaves .....	0.003	0.15	trace		0.15	1.07
Roots .....	0.020		0.07		0.14	
Low K						
Stems, leaves .....	0.005	0.20	0.05	0.30	0.19	0.82
Roots .....	0.022		0.17		0.23	
Medium K						
Stems, leaves .....	0.005	0.20	0.05	0.45	0.14	0.87
Roots .....	0.023		0.11		0.16	
High K						
Stems, leaves .....	0.005	0.20	0.05	1.00	0.17	1.06
Roots .....	0.024		0.05		0.16	

Adding Na to the graphic presentation of interrelated antagonisms given previously suggests the following relationship:



It is difficult to explain why the absorption of Na is dominated by Ca and K. It is possible that the greater hydrolyzation of Na salts plays a part. It is possible also that after Ca and K have entered the stems and leaves they may exercise their antagonistic action toward Na. It is doubtful that insoluble compounds of Na accumulate in the roots.

Phosphorus differs from the other elements discussed in that it is absorbed as an anion. The P content per unit of dry weight varies somewhat, yet its "T<sub>Q</sub>" appears to be much more significant, varying directly with Ca and to a lesser degree, with K. It appears that Ca facilitates the translocation of P. When K is present in greater abundance, it likewise aids somewhat in this function.

### Discussion

In figure 7 are given the smoothed curves of absorption of K, Ca, and Mg

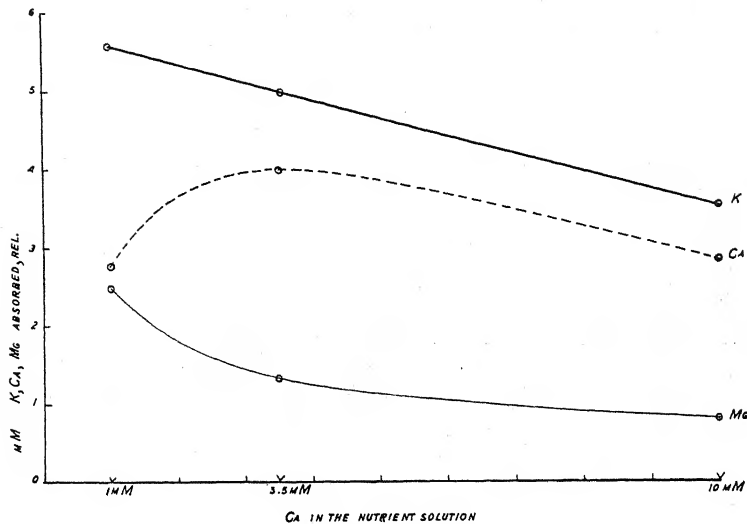


Fig. 7. Smoothed absorption curves of potassium, calcium, and magnesium with varying supply of calcium in the nutrient solution.

in the two experiments with varying Ca in the nutrient solution. With increasing Ca supply, K decreases per unit dry weight in a straight line curve. The curve for the variable Ca at first increases and later parallels the K curve. When it is recalled that total absorption of Ca parallels growth, it suggests that this element fills a function closely related to the growing processes, *e.g.*, cell division. Ca was stored in the tissues in proportion to

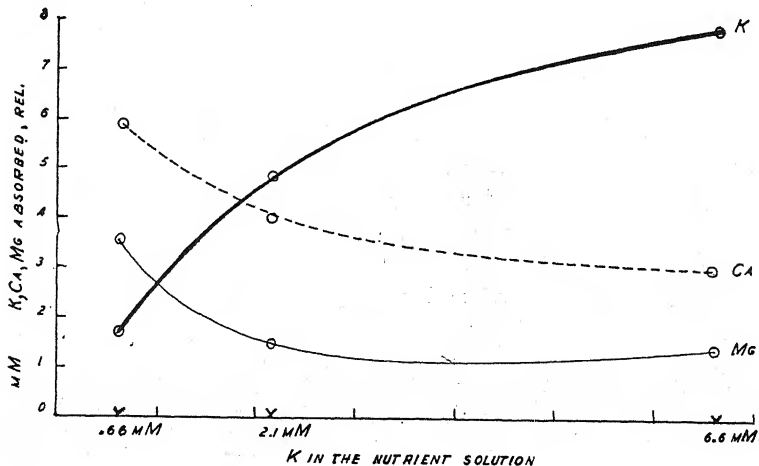


Fig. 8. Smoothed absorption curves of potassium, calcium, and magnesium with varying supply of potassium in the nutrient solution.

the tissues formed. In the absence or insufficient supply of Ca, cells at the heliotropic or geotropic growing points fail to develop.

Magnesium decreases in the plant as the Ca concentration is increased in the nutrient solution. The trend of the curve, however, seems to indicate that the greater the outer concentration of Ca, the less depressing is the effect on Mg; this is apparently a result of decreased inner concentration of Ca.

In figure 8 are shown the smoothed curves of absorption of K, Ca and Mg under varying concentrations of K in the nutrient solution. Potassium is absorbed somewhat in proportion to K present in the solution, while Ca and Mg are decreased.

Any study of base absorption and translocation in plants must consider antagonism. BURSTRÖM (2) in studying cation absorption in oats defines "antagonism" as the ability of one ion to retard the absorption of another. RUBINSTEIN (13) recognizes a "pseudo-antagonism" for cases of apparent antagonism due to difference in ion concentration. The writer believes that a true picture of antagonistic phenomena cannot be correctly estimated without considering both categories. It should also be considered what forces or conditions govern antagonism and pseudo-antagonism which must necessarily be operative in the root zone. In antagonism where the force or nature of one ion is matched with one of different character, it is evident that the permeability of the plasma membrane of the outer root cells is involved. LUNDEGÅRDH (6) has pointed out that this permeability to elements (ions) in solution stands in a direct relation to the diameter of the ions and to a certain extent to their specific mobility. Furthermore, the degree of hydrolyzation of inorganic ions is a factor, since they vary in their affinity to molecules of water. This affinity increases with valence and decreases with size of ions. Thus, K ions possess a greater mobility than Ca ions and the latter a greater mobility than Mg ions. It is concluded therefore that the cause of antagonism is due to variation in mobility and size of ions, and to a certain degree—hydrolyzation.

Pseudo-antagonism, evident in the end result in the same way as antagonism, operates through the force of mass action, and is not a matter of ion equivalence but of a difference in ion concentration. The operation of mass action thus produces an apparent antagonism, or more properly, a pseudo-antagonism. It is clear that both these categories, due to different causes, warrant consideration in a discussion of ion absorption. The curves presented in figures 7 and 8 support the views given above.

In the present work the evidence confirms the findings of other investigators (9, 11) that Mn is essential for normal growth and development of plants. The absorption of K, Ca, and Mg in the presence and absence of Mn has been discussed under the individual experiments. It may be of interest, however, to view the relative absorptions of K and Ca, *i.e.*, in percentages

of quantities supplied in the nutrient solutions with and without Mn. A graphical summary is given in figure 9.

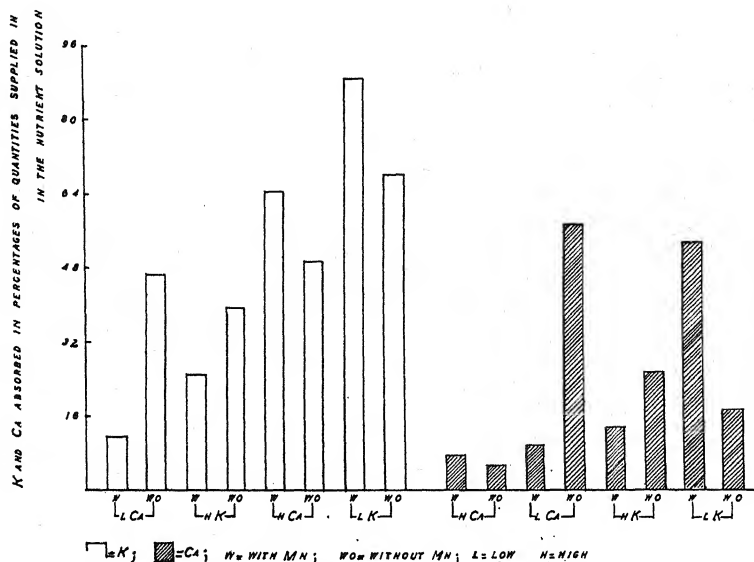


FIG. 9. Graphical summary of potassium and calcium absorption in percentages of quantities supplied in the nutrient solution, with and without addition of manganese.

The efficiency of K utilization was greater in absence of Mn at "low Ca" and "high K." The reverse is true at "high Ca" and "low K." Where Ca dominated, Mn facilitated the utilization of K, which was adversely affected when K dominated. The greatest relative utilization occurred at low-K supply in the presence of Mn.

In viewing Ca absorption, it is evident that where K is dominating ("low Ca," "high K"), utilization of Ca is improved in the absence of Mn. Where, however, Ca dominated ("high Ca," "low K") addition of Mn benefitted the utilization of Ca. The highest efficiency of Ca utilization was derived from a low-Ca supply without Mn. Therefore, it is concluded that Mn retards the absorption of Ca, when pseudo-antagonism is operative on the Ca ions.

From a practical viewpoint, Mn is of greater importance in soils where Ca dominates (a high-Ca level or a relatively low level of K) than where Ca is deficient. Applications of Ca (lime) should be guided by the actual content of active Mn in the soil (4). In Rhode Island (3) where soils are generally low in Mn, crops have shown symptoms of Mn deficiency through excessive liming.

On the other hand, in the tobacco growing district of Connecticut where soils are kept rather acid, the problem of excessive absorption of Mn is a serious one since a content of more than 0.03 per cent. Mn per unit dry

weight of the leaf causes discoloration of the ash, known to the cigar trade as muddy or brick colored ash (1).

The diagram in figure 10 shows the absorption of Ca in percentages of Ca supplied in presence and absence of B. Most striking is the treatment "low K." The lowest efficiency obtained in the utilization of Ca occurred in the absence of B while the highest occurred with boron present. Here Ca was dominating as in "high Ca," where B had little effect on Ca utilization. This may mean that B produces a pseudo-antagonism to Ca. Where this is

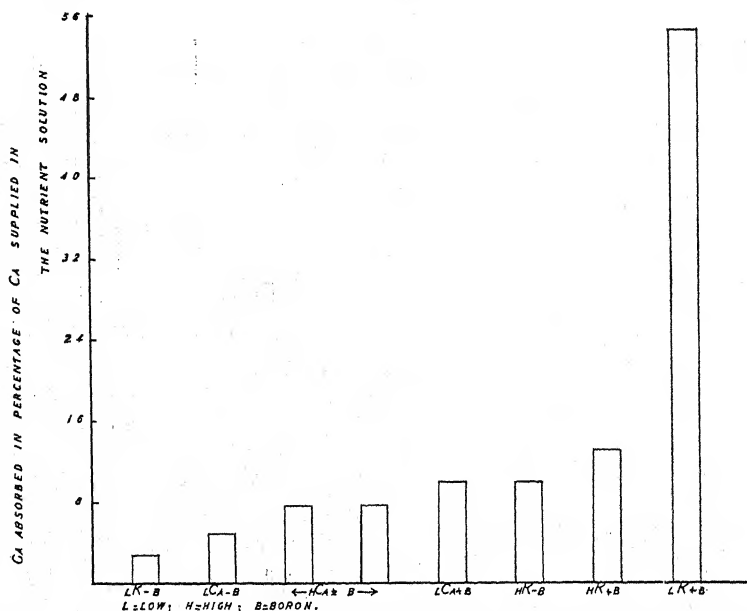


FIG. 10. Graphical summary of calcium absorbed in percentage of calcium added to the nutrient solution in the presence or absence of boron.

strong enough, addition of B does not improve the efficiency of Ca. This, however, should not be confused with the positive physiological need of B. Moreover, where Ca was physiologically deficient ("low Ca," "high K") the presence of B facilitated the utilization of Ca. It is concluded, therefore, that B aids the absorption of Ca. The practical aspect of this finding is the application of B to soils where Ca absorption needs to be improved, that is, where K, Mg, H, and  $\text{NH}_4$  (7) or other ions exert antagonism or pseudo-antagonism sufficiently to limit Ca absorption below growth requirements. In line with this are the observations of McMURTREY (10) on B deficiency symptoms of tobacco under field conditions.

There is some resemblance between Ca deficiency and B deficiency symptoms of tobacco in the injury to the terminal bud, although McMURTREY (8) states that lack of boron affects the bases of the young leaves, while tops and margins are affected by lack of Ca. The writer has observed, however, that

die-back of the mid-vein at the base also occurs on Ca deficient tobacco plants upon longer standing. WARINGTON (15) has shown that a close association exists between Ca and B in the absorption and utilization of Ca. It is likely that a close association also prevails within the plant. From the work by JOHNSTON and DORE (5) it may be inferred that the production of pectic substances may be related to the presence of B. They state that B is apparently essential to cell division, to which function the presence of Capectate is also related in the wall formation (12). Further studies are required to elucidate the definite relationship between Ca and B.

In this paper the expression "Translocation Quotient" (Tq) has been introduced. It expresses the ratio between ions present in stems and leaves (per unit dry weight) and identical ions found in the roots. It would appear that the use of a quotient or ratio might aid in any interpretation of data. The nearest approach to a "Tq" is BURSTRÖM'S (2) "total absorption per unit root weight," employed in the present work for computations involved in figures 2 and 4. While this latter calculation is useful to establish antagonism, simpler calculations shown in this paper have served a similar purpose and perhaps more convincingly. The "Tq" may be useful in studies of rate of growth where magnitude and variation in treatment are compared in order to observe cation as well as anion translocation.

### Summary

In this paper work has been reported on absorption of bases (mainly the three cations K, Ca, Mg) studied by means of water cultures. Tobacco of a type commonly grown in the Connecticut Valley (Havana Seed) was used as a test plant. Moreover, the interrelated antagonistic effect of these ions were observed. A distinction between antagonism and pseudo-antagonism was made, and the reason for it outlined. It was shown that, while K is antagonistic to Ca, this ion under certain conditions may effect a pseudo-antagonism toward K. The same is true with Ca versus Mg.

The effect of varying quantities of K and Ca in the nutrient solution on the absorption of Fe, Na and P was also observed. Ca depresses the uptake and translocation of Fe and is antagonistic to Na. The translocation of P is aided by Ca.

The absorption of the three cations was also studied in the absence and presence of B and Mn in the nutrient solutions. In general it was found that B aids absorption and utilization of Ca.

Mn under certain conditions has a regulating influence on the absorption of Ca, while this ion *per se* is antagonistic to Mn.

A "translocation quotient" (Tq) has been suggested in this paper to be used in connection with absorption data in order to facilitate interpretation of translocation phenomena.

This work was conducted in the laboratories of Professor H. LUNDE-

GÄRDH, Centralanstalten, Stockholm, Sweden. The author is greatly indebted to Professor LUNDEGÄRDH and his associate, Dr. HANS BURSTRÖM, for use of laboratory and equipment as well as valuable suggestions pertaining to this study.

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# TRANSPIRATION AND PHYSICO-CHEMICAL PROPERTIES OF LEAVES AS RELATED TO DROUGHT RESISTANCE IN LOBLOLLY PINE AND SHORTLEAF PINE<sup>1</sup>

C. S. SCHOPMEYER

(WITH TWO FIGURES)

## Introduction

In the reforestation program now being carried on by various state and federal agencies, many millions of tree seedlings are planted each year. An extremely large number of these planted seedlings are killed within the first year or two after planting. One of the major causes of the fatalities is the inability of seedlings to resist drought. If the properties involved in drought resistance of seedlings were known, and if those species and age classes of planting stock possessing such properties could be identified or developed, much better survival could be obtained in plantations of tree seedlings.

Drought resistance, the ability of a plant to endure permanent wilting without harm to subsequent development (11), has been induced in plants by repeated wilting and also by growing them in soil with a moisture content barely above the wilting percentage (27, 28). Observations on permanently wilted herbaceous plants (27) show that their transpiration rate may be less than one-fourth of the rate for turgid plants. Most conifers, however, do not show any external evidence of a wilted condition. Hence, in hardening conifers against drought, the wilting percentage of soil moisture must be previously determined to facilitate control of soil moisture conditions.

Although attempts have been made to correlate drought resistance with rate of transpiration, there is much evidence (1, 2, 4, 21, 24, 32) to support the statement of MAXIMOV and KRASNOSSELSKY-MAXIMOV (12) that the rate of transpiration when soil moisture is abundant cannot be used for judging drought resistance. Transpiration may be greatly reduced when soil moisture approaches the wilting percentage (15, 19) and the reduction may vary among plants of different morphological structure, but there appears to be no consistent relationship between drought resistance and rate of transpiration at soil moisture contents near the wilting percentage.

There is evidence of a correlation between drought resistance and bound water content of leaf tissue. KORSTIAN (9) found some indication of such a correlation in a preliminary study of southeastern tree species. The work of NEWTON and MARTIN (17) is particularly outstanding in that a very definite correlation was obtained in cereal crops and grasses between the degree of drought resistance and the bound water content. NOVIKOV (18) also

<sup>1</sup> This investigation was supported by a Fellowship in Forestry in the Graduate School of Arts and Sciences of Duke University.



found that the amount of bound water present characterized the degree of drought resistance in varieties of wheat when they were in a wilted state but not in a turgid state. He attributed an observed increase in bound water during hardening not to a decrease in total water but to physico-chemical changes in the cell contents.

Information on the relationship of osmotic pressure of cell sap to drought resistance is very conflicting. DRABBLE and DRABBLE (3) and LIVINGSTON (10) have shown that a high osmotic pressure cannot lower the vapor pressure of cell sap sufficiently to reduce water loss by transpiration to an appreciable extent. KORSTIAN (8) obtained evidence indicating that water absorption by plants depends on osmotic pressure, but STODDART (25) believes that a high osmotic pressure is a result of drought and not an adaptation to it. NEWTON and MARTIN (17) found that osmotic pressure was not a consistently reliable index of drought resistance. In comparing osmotic pressures of two or more species it is difficult to make a proper interpretation because in most cases it is not known whether differences are caused by different water contents or by different solute concentrations.

The primary purpose of this investigation was to ascertain whether the relative drought resistance of seedlings of two species can be determined by physico-chemical means. A secondary purpose was to obtain information on the behavior of seedlings during and after drought by a study of changes in physico-chemical properties. The approach was made by a study of transpiration, bound-water content, total water content, osmotic pressure, and calculated solute concentration in leaves of two species growing in a greenhouse under controlled soil moisture conditions. Loblolly pine (*Pinus taeda* L.) and shortleaf pine (*P. echinata* Mill.) were selected for comparison because both species occur within the same region with loblolly pine on mesic sites, and shortleaf pine on more xeric sites. The apparent adaptations of these species to different sites indicate that shortleaf pine is the more drought resistant of the two species.

## Materials and methods

### SEEDLINGS AND SOIL

A group of vigorous one-year-old seedlings of loblolly pine and of shortleaf pine were selected at the North Carolina State Nursery and moved to a greenhouse in December, 1935. The seedlings were planted in two-gallon buckets containing Congaree silt loam, the moisture constants of which are presented in table I. A watering tube extended down the side of each bucket into a layer of crushed rock in the bottom. The buckets were covered with a double layer of oilcloth to prevent evaporation of water from the soil. Soil moisture was maintained at 30 per cent. for a month after the completion of planting to permit the seedlings to become established.

TABLE I  
MOISTURE CONSTANTS FOR CONGAREE SILT LOAM

ITEM	MOISTURE CONTENT (PERCENTAGE OF DRY WEIGHT)
	%
Moisture equivalent .....	27.3
Wilting coefficient .....	14.8
Wilting percentage .....	7.7
Saturation percentage .....	53.3

#### DETERMINATION OF TRANSPIRATION

The gravimetric method for the determination of transpiration was selected for this work. The buckets were weighed daily to determine the weight of water lost by transpiration which was expressed in grams per gram of oven-dried leaf tissue per day.

On April 4 the seedlings of each species were divided into three groups for a study of transpiration under three conditions of soil moisture. In the first group of seedlings, the soil moisture was brought to 30 per cent. at weekly intervals. This moisture content is believed to be approximately an optimum for growth. Between waterings the soil moisture in this group rarely went below 25 per cent. In the second group, the seedlings were left unwatered, and the soil moisture was gradually decreased to the wilting coefficient. Approximately six weeks elapsed before the wilting coefficient was reached. This treatment, according to TUMANOV (27), should induce drought hardening. In the third group, the soil moisture was allowed to reach the wilting coefficient, as in the second group, and was then brought back to 30 per cent. moisture. The latter treatment enabled the seedlings to recover from the effects of drought and to resume growth. In the first two groups, transpiration determinations were made at weekly intervals. In addition, daily determinations were made near the end of the experiments for all three groups.

#### DETERMINATION OF PHYSICO-CHEMICAL PROPERTIES

After completing the transpiration determinations ten samples were collected from each species in each of the three soil moisture categories. Collections were made at 11 P.M. One sample consisted of all the leaves from one seedling. The fresh weight of the sample was obtained after placing it in a closed tin container. Each sample was macerated in a meat grinder and divided into three parts for the determination of bound water, total water, and freezing point, respectively.

The calorimetric method for the determination of bound water (5, 13, 16, 20, 22, 23, 26) was selected for this work. In making the determinations, ten

samples from each category were run through the procedure together. The samples, each consisting of about 8 grams of the macerated leaf tissue, were placed in tinfoil cups similar to those used by ROBINSON (20) and GREATHOUSE (5). The cups were placed in glass vials, tightly stoppered, and weighed to  $\pm 0.001$  gram. After weighing, the tinfoil cups containing the samples were placed in stoppered freezing tubes with two samples in each tube. The freezing tubes were suspended in a freezing bath at a temperature of  $-20^{\circ}\text{C.} \pm 2^{\circ}\text{C.}$  through holes in a cover over the bath. The samples were left in the bath for approximately 12 hours. A pint thermos bottle, containing 250.0 gm. of water was used for a calorimeter. The initial temperature of the water in the calorimeter was determined by using a mercury thermometer which was read to  $\pm 0.005^{\circ}\text{C.}$  A sample, enclosed in its tinfoil container, was transferred from the freezing bath to the calorimeter. After continuous stirring with a motor-driven stirring rod for approximately 8 minutes, thermal equilibrium was reached and the final temperature was recorded. A correction factor for the heat absorbed by the calorimeter and accessories was obtained by placing 8 ml. of water in the tinfoil cups and following the procedure just outlined. The formulae used in calculating the bound water contents are essentially the same as those used by GREATHOUSE (5).

In preparing samples for specific heat determinations on the dry matter in the leaf tissue, leaves were dried for 24 hours at  $95^{\circ}\text{C.}$  and then ground to a fine dust with a mortar and pestle. The ground leaf tissue was placed in tinfoil cups and dried to constant weight  $\pm 0.001$  gram at  $95^{\circ}\text{C.}$  The specific heats were determined using the same procedure as for bound water except that benzene was used instead of water in the calorimeter. Benzene, having a lower specific heat, facilitated measurement of the temperature change in the calorimeter. The specific heats of benzene and of tin were obtained by plotting their values as given in the *International Critical Tables* (31) and then interpolating for the desired temperature. The specific heats of water and of ice were obtained from the *Handbook of Chemistry and Physics* (7). For loblolly pine the mean of 5 specific heat determinations on the dry leaf tissue was 0.213 with a standard error of  $\pm 0.002$ ; the mean of 5 determinations on shortleaf pine was 0.208 with a standard error of  $\pm 0.006$ .

The total water content of a sample was assumed to be the difference between the fresh weight and the weight after drying the macerated tissue to constant weight  $\pm 0.001$  gm. at  $95^{\circ}\text{C.}$  The water content was calculated on a dry weight basis.

Freezing point determinations were made on the leaf tissue using the thermo-electric method (14). The apparatus consisted of two thermocouples connected in series to a galvanometer. One junction was maintained at  $0^{\circ}\text{C.}$  The other junction was sealed into the tip of a hypodermic needle

and inserted into a 1.5-cm. length of glass tubing containing a portion of the macerated leaf tissue. The junction with the enclosing sample was placed in a freezing tube suspended in a freezing bath. The amount of undercooling and the observed freezing point were recorded. The true freezing point and the osmotic pressure were calculated from the tables of HARRIS and GORTNER (6).

Since osmotic pressure of a tissue varies with total water content, the usefulness of osmotic pressure for comparisons of solute concentration in tissues of different water contents is very limited. In an attempt to obtain a more stable factor for such comparisons, the following conversion was devised.

$$\frac{\pi \times [\text{H}_2\text{O}]}{22.4} = [\text{Solute}]$$

where

$\pi$  = osmotic pressure

$[\text{H}_2\text{O}]$  = grams of water per gram of dry leaf tissue

$[\text{Solute}]$  = number of gram molecular weights (mols) of solute per kilogram of dry leaf tissue

22.4 = the osmotic pressure of a mol of undissociated solute in 1000 grams of water

This conversion does not give a true measure of solute concentration because the relative amounts of ionized and un-ionized solutes are not known. This formula merely converts osmotic pressure to its equivalent in mols of undissociated solute on a dry weight basis and thus gives a factor which probably does not vary to a great extent with changes in water content. This factor will be designated as *solute concentration* in the following discussion, but

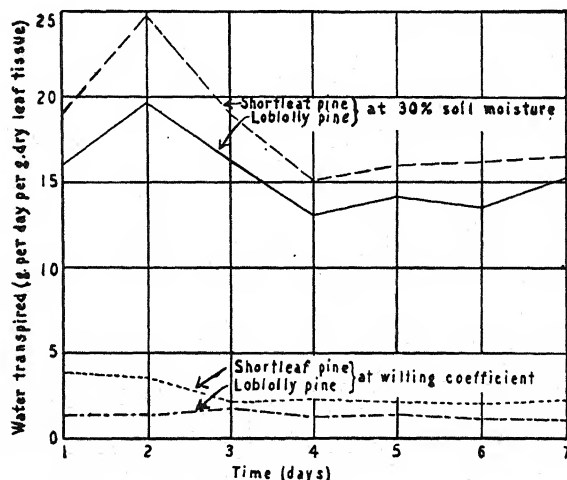


FIG. 1. Daily transpiration of loblolly pine and shortleaf pine at 30 per cent. soil moisture and at the wilting coefficient. Each point is the mean value of 9 determinations.

actually it is just a rough approximation to the true solute concentration because of the unknown degree of dissociation.

## Results and discussion

### TRANSPIRATION

Shortleaf pine had a higher daily rate of transpiration than loblolly pine with soil moisture at 30 per cent. and also at the wilting coefficient. Mean values of nine trees of each species in the two soil moisture categories on seven successive days are shown in figure 1. The differences between the two species are highly significant at both soil moisture contents as shown by the analysis of variance in table II. Significant also is the variation in

TABLE II

ANALYSIS OF VARIANCE IN TRANSPIRATION RATES OF LOBLOLLY PINE AND SHORTLEAF PINE

SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	CALCULATED <i>F</i> VALUE
Soil moisture at 30 per cent.				
Species .....	1	222.60	222.60	59.52†
Days .....	6	915.56	152.59	40.80†
Interaction .....	6	45.30	7.55	2.02*
Error .....	112	419.35	3.74	
Total .....	125	1602.81		
Soil moisture at wilting coefficient (14.8 per cent.)				
Species .....	1	41.18	41.18	76.26†
Days .....	6	16.99	2.83	5.24†
Interaction .....	6	16.06	2.68	4.96†
Error .....	112	60.27	0.54	
Total .....	125	134.50		

\* Not significant (less than 5 per cent. point).

† Highly significant (greater than 1 per cent. point).

transpiration rates resulting from the variation caused by differences in temperature and humidity from day to day. These results indicate that the apparently greater ability of shortleaf pine to resist drought cannot be attributed to an ability to conserve water by retarding transpiration. This conclusion agrees with the statement of MAXIMOV (11) that xeric plants are distinguished by a higher rate of transpiration than that of more mesic plants.

The rate of transpiration of loblolly pine gradually diminished over a period of six weeks as the decreasing soil moisture approached and passed the wilting coefficient (14.8 per cent. moisture). At the end of the six-week period, the rate was only 15.8 per cent. of that at 30 per cent. soil moisture. This gradual reduction is not to be expected according to the results of VIEHMEYER and HENDRICKSON (30). They found that extraction of water

from soil by peach trees is not affected by soil moisture content until it is reduced to the wilting percentage. Hence transpiration would be expected to remain constant until the wilting percentage is reached. The gradual reduction observed in this work can be explained, however, by assuming that at the beginning of the period of drought, only a portion of the roots were in soil at the wilting coefficient; and as more and more of the roots extracted all of the water available to them, transpiration was gradually reduced. The transpiration rates of the two species at the wilting coefficient, shown in figure 1, are not as low as the negligible amounts of water lost by western conifers as observed by PEARSON (19). The calculated wilting coefficient for the soil used in this study, however, as shown in table I, is approximately 7 per cent. higher than the true wilting percentage as determined experimentally. Hence there may have been an appreciable amount of available soil moisture at the calculated wilting coefficient.

With soil moisture at 30 per cent. after having been reduced to the wilting coefficient, differences in transpiration rates between the two species were not significant. A comparison of the transpiration rates of loblolly

TABLE III

ANALYSIS OF VARIANCE IN BOUND WATER, TOTAL WATER, OSMOTIC PRESSURE, AND SOLUTE CONCENTRATION IN LEAF SAMPLES OF LOBLOLLY PINE AND SHORLEAF PINE COLLECTED AT 11 P. M.

ITEM	SOURCE OF VARIATION	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARE	CALCULATED <i>F</i> VALUE
Bound water	Species	1	109.54	109.54	1.65*
	Soil moisture	2	1274.29	637.65	9.61†
	Interaction	2	129.87	64.94	0.98*
	Error	54	3581.43	66.32	
	Total	59	5095.13		
Total water	Species	1	1920.6	1920.6	10.55†
	Soil moisture	2	24202.3	12101.2	66.49†
	Interaction	2	187.1	93.6	0.51*
	Error	54	9827.2	182.0	
	Total	59	36137.2		
Osmotic pressure	Species	1	66.03	66.03	51.19†
	Soil moisture	2	540.34	270.17	209.43†
	Interaction	2	129.06	64.53	50.02†
	Error	54	69.57	1.29	
	Total	59	805.00		
Solute concentration	Species	1	1.3735	1.3735	88.61†
	Soil moisture	2	3.1324	1.5662	101.05†
	Interaction	2	0.6717	0.3358	21.66†
	Error	54	0.8373	0.0155	
	Total	59	24203.0	12101.0	66.49†
			6.0149		

\* Not significant.

† Highly significant.

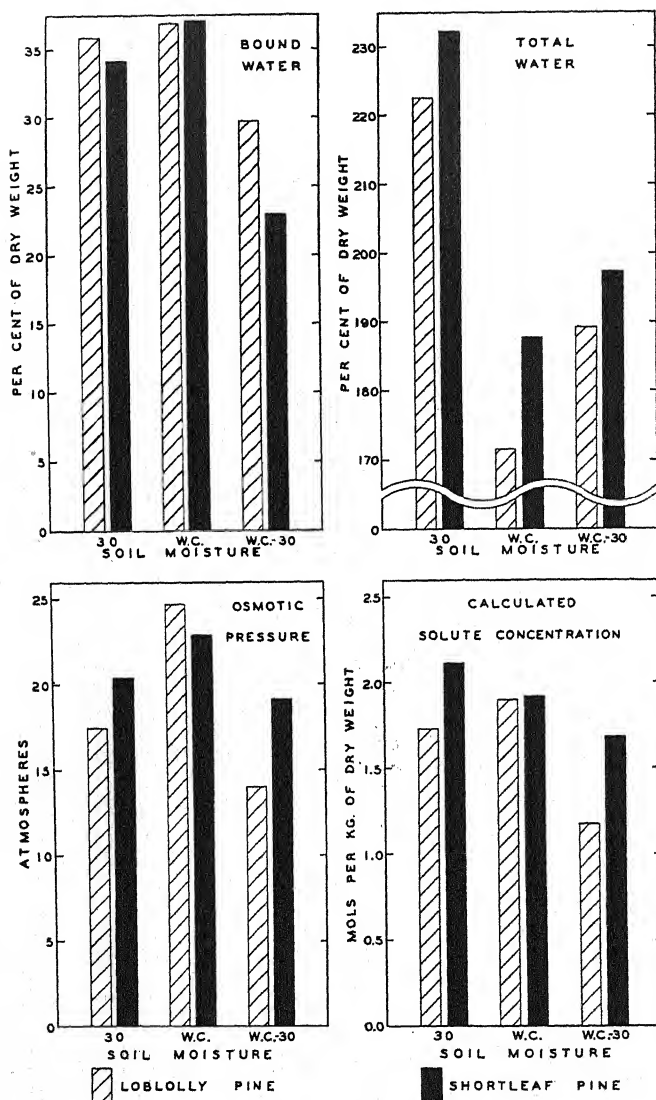


FIG. 2. Mean values of bound water, total water, osmotic pressure, and solute concentration in leaves of loblolly pine and shortleaf pine under various soil moisture conditions. Each value is the mean of 10 determinations.

30 = Soil moisture maintained continuously at 30 per cent.

W.C. = Soil moisture at the wilting coefficient.

W.C.-30 = Soil moisture at 30 per cent. after having been reduced to the wilting coefficient.

TABLE IV  
SIGNIFICANCE OF DIFFERENCES BETWEEN MEANS OF PHYSICO-CHEMICAL PROPERTIES OF LEAVES OF LOBLOLLY PINE AND SHORTEAF PINE

CATEGORIES COMPARED*	BOUND WATER		TOTAL WATER		OSMOTIC PRESSURE		SOLUTE CONCENTRATION	
	DIFFERENCE BETWEEN MEANS	t VALUE†	DIFFERENCE BETWEEN MEANS	t VALUE†	DIFFERENCE BETWEEN MEANS	t VALUE†	DIFFERENCE BETWEEN MEANS	t VALUE†
P:30-LP:30 .....	%		%		Atm.		Mols.	
P:WC-LP:WC .....	-1.76	0.5	9.66	1.5	2.98	5.8	0.384	8.3
P:WC:30-LP:WC:30 .....	0.36	0.1	16.23	2.7	-1.85	3.4	0.016	0.3
P:30-LP:WC .....	-6.71	1.8	8.06	1.3	5.17	10.1	0.512	11.1
P:30-LP:WC:30 .....	-1.09	0.3	51.02	8.5	-7.26	14.2	-0.168	3.6
P:WC-LP:WC:30 .....	6.06	1.7	33.37	5.5	3.46	6.8	0.556	12.1
P:WC-LP:WC:30 .....	7.15	2.0	-17.65	2.9	10.72	21.0	0.724	15.7
P:30-SP:WC .....	-3.01	0.8	44.45	7.4	-2.42	4.8	0.200	4.3
P:30-SP:WC:30 .....	11.16	3.1	34.97	5.8	1.28	2.5	0.438	9.3
P:WC-SP:WC:30 .....	14.13	3.9	-9.47	1.5	3.70	7.3	0.229	5.0

\* SP = shortleaf pine. LP = loblolly pine.  
 30 = samples from trees in soil at 30 per cent. moisture.  
 WC = samples from trees in soil with moisture content at the wilting coefficient.  
 WC:30 = samples from trees in soil at 30 per cent. moisture after having been reduced to the wilting coefficient.  
 † t = difference between means. Values of t greater than 2.0 are considered significant.  
 standard error of difference



pine supplied continuously with 30 per cent. soil moisture and of the same species at 30 per cent. soil moisture after having been reduced to the wilting coefficient showed no significant difference. No comparison was made on the transpiration rates of seedlings of shortleaf pine at these two soil moisture contents.

#### PHYSICO-CHEMICAL PROPERTIES OF LEAF TISSUES

Analyses of variance were made on all physico-chemical data and are presented in table III. These analyses show that total water, osmotic pressure, and solute concentration in leaves of shortleaf pine and loblolly pine differed significantly between species as well as between soil moisture categories. The analyses also show that soil moisture treatments caused significant differences in bound water contents, and that species did not. The mean values of each physico-chemical property in each category are presented graphically in figure 2. Tests of the significance of differences between individual means are presented in table IV.

The bound water content values may be subject to criticism because of the large variation within categories as indicated by the magnitude of the mean square of error in the analysis of variance in table III. Two factors may contribute to this variation: first, inconsistencies in the technique used in making the determinations; and second, variation in the amount of bound water from tree to tree within categories. No replicated bound water determinations were made on substances known to be homogeneous; hence an absolute check on the reliability of the method is not available. The replications of the specific heat determinations, however, and the calorimeter factor determinations, presented in table V, are very consistent. Since these deter-

TABLE V

COMPARISON OF VARIATION IN REPLICATED DETERMINATIONS OF BOUND WATER, SPECIFIC HEAT, AND THE CALORIMETER FACTOR

ITEM	SPECIFIC HEAT OF LOBLOLLY PINE	CALORIMETER FACTOR	BOUND WATER*
Number of replications .....	5	4	10
Mean .....	0.213	1.070	35.81
Standard deviation .....	0.00336	0.00895	7.78
Standard error .....	0.00151	0.00448	2.46
Standard error expressed as a percentage of the mean .....	0.7	0.4	6.9

\* Determinations were made on leaves of loblolly pine when soil moisture was at 30 per cent.

minations were made using the same apparatus that was used in making the bound water determinations, the consistency of the specific heat values and the calorimeter factors is an indication of the reliability of the technique.

When soil moisture was at 30 per cent., leaves of shortleaf pine were higher in total water, osmotic pressure, and solute concentration than were leaves of loblolly pine. Bound water contents were not significantly different in leaves of the two species. Consideration of these data alone might lead to the conclusion that the greater drought resistance of shortleaf pine is attributable to osmotic effects. Data on the physico-chemical properties under the other two soil moisture conditions show, however, that osmotic effects have no direct connection with the relative drought resistance of loblolly pine and shortleaf pine.

Changes in the seedlings which occurred when soil moisture was reduced from 30 per cent. to the wilting coefficient furnish some interesting information on the behavior of the two species during drought hardening. Seedlings of both species became dormant as judged by the cessation of growth and the setting of terminal buds. In leaves of both species osmotic pressure increased, and total water decreased considerably. Solute concentration apparently decreased in leaves of shortleaf pine, but increased in leaves of loblolly pine. These changes in solute concentration, although small, are statistically significant. They are not believed to be physiologically significant, however, because of the fact that the changes are in opposite directions in the two species.

No significant change occurred in the bound water content of the leaves of either species when soil moisture was reduced from 30 per cent. to the wilting coefficient. This fact leads to the assumption that no appreciable change took place in the amount of water-binding colloids and crystalloids in leaves of the two species during the drought hardening period. The fact that no consistent change in solute concentration occurred in the two species when soil moisture was reduced may be regarded as evidence partially supporting this assumption.

Physico-chemical differences between species when soil moisture was at the wilting coefficient show which properties cannot be used for determining the relative drought resistance of the two species and also show which factors might be indicative of the relative drought resistance. With soil moisture at the wilting coefficient, leaves of shortleaf pine had a higher total water content and a lower osmotic pressure than leaves of loblolly pine. Solute concentration and bound water contents were practically the same in both species.

Since solute concentration on a dry weight basis was the same in the two species when soil moisture was at the wilting coefficient, it follows that the observed difference in osmotic pressure was the result of the difference in total water content. Since osmotic pressure was influenced in this way, it cannot be used as an indicator of the relative drought resistance of the two species.

The simultaneous occurrence of a higher total water content and a higher transpiration rate in leaves of shortleaf pine when soil moisture was at the wilting coefficient, indicates that this species had a faster rate of absorption of water from soil than loblolly pine when soil moisture was limited. This ability of shortleaf pine might be attributed to a lower ratio of evaporating leaf surface to absorbing root surface. A superficial examination of the root systems of the seedlings used in this work indicated that shortleaf pine seedlings had a lower top-root ratio than loblolly pine. More exact measurements are necessary, however, to prove the point.

The ability of a seedling to maintain a higher total water content in its leaves when soil moisture is limited may be an aid to survival during periods of drought in that it might prolong the period of permanent wilting and thus delay the occurrence of a water content in the leaves below the minimum necessary for life. Such a prolongation of the period of permanent wilting, however, would enable a seedling to survive periods of drought only as long as the soil moisture content did not go below the wilting percentage for a prolonged period.

The fact that shortleaf pine, the more drought resistant of the two species studied, had the greater total water content in its leaves when soil moisture was at the wilting coefficient, leads to the conclusion that the magnitude of the total water content may be used as an indicator of the relative drought resistance of the two species under the conditions of this experiment.

In view of the correlation obtained by other workers between bound water contents and relative drought resistance, an explanation of the lack of such correlation in this study, when soil moisture was at the wilting coefficient, is pertinent. NEWTON and MARTIN (17) who obtained a good correlation between drought resistance and bound water contents of cereal crops and grasses, used the cryoscopic method for the determination of bound water in expressed sap. With this method, only the amount of bound water held by the colloids, ions, and molecules expressed in the sap is determined. MEYER (13) stated that a considerable amount of water may be bound in cell walls of plant tissues. Since leaves of many species of the genus *Pinus*, including loblolly pine and shortleaf pine, are characterized by a hypodermal layer of cells with thick walls, the amount of water bound by dispersed substances within the cells may be negligible compared to the amount of water bound in the cell walls. In this investigation, bound water determinations were made on macerated leaf tissue which included both cell walls and cell contents. The amount of water-binding surface in cell walls per unit weight of dry leaf tissue may have been sufficiently uniform in the two species to prevent the detection of small differences in the amount of bound water in the cell sap. Since no data are available on the relative amounts of bound water in cell walls and in cell sap, any interpretation of

the bound water contents of the leaf tissue must be based on their face value. Hence, the magnitude of the bound water content of the leaf tissue as determined in this investigation, is not a factor which makes shortleaf pine more drought resistant than loblolly pine.

Striking changes occurred in the leaves of the two species after soil moisture was increased from the wilting coefficient to 30 per cent. The physico-chemical properties were determined one week after the soil moisture had been brought back to 30 per cent. During that week dormancy was broken and growth was resumed. Total water contents of leaves were greater than when soil moisture was at the wilting coefficient, but less than when seedlings were first supplied with 30 per cent. soil moisture. Large decreases in bound water, osmotic pressure, and solute concentration occurred in leaves of both species. Each of these three properties were lower in magnitude than in either of the other two soil moisture categories. These results agree with those of VASSILIEV and VASSILIEV (29) who found that wheat plants grown in soil with insufficient water and then watered abundantly were lower in water content as well as in monosaccharides and sucrose than check plants grown continuously with abundant soil moisture.

Total water, osmotic pressure, and solute concentration were higher in leaves of shortleaf pine than in leaves of loblolly pine after 30 per cent. soil moisture was restored. No significant difference in the bound water contents of the two species was observed in this soil moisture category.

The decreased solute concentration and the decreased bound water contents in the leaves of the two species indicate a rapid depletion of food reserves, including water-binding colloids, in the leaves when dormancy resulting from drought is broken by the restoration of abundant soil moisture. The fact that the decrease in solute concentration was not as great in shortleaf pine as in loblolly pine indicates that the former species may have maintained a better balance between utilization and synthesis of soluble food reserves. This closer balance may enable shortleaf pine to recover more rapidly from the effects of drought, and hence it may be a factor contributing to the ability of this species to survive on drier sites than those on which loblolly pine occurs.

### Summary

Determinations of transpiration, bound water, total water, and osmotic pressure were made on seedlings of loblolly pine and shortleaf pine to obtain information on the behavior of these species during and after drought and to ascertain whether any of these factors can be used as indicators of relative drought resistance. Solute concentration, calculated from data on osmotic pressure and total water content, was a valuable aid in interpreting the osmotic pressure differences.

With soil moisture at 30 per cent., shortleaf pine had a higher transpira-

tion rate, more total water, a higher osmotic pressure, and a greater solute concentration than loblolly pine. Bound water contents were not significantly different in the two species.

With soil moisture at the wilting coefficient, shortleaf pine had a higher transpiration rate, more total water, and a lower osmotic pressure than loblolly pine. Solute concentration and bound water contents were practically the same in both species.

When soil moisture was restored to 30 per cent. after having been reduced to the wilting coefficient, leaves of shortleaf pine had more total water, a greater osmotic pressure and a higher solute concentration than leaves of loblolly pine. Transpiration rates and bound water contents were practically the same in both species.

Bound water contents decreased in both species when soil moisture was restored to 30 per cent. after having been reduced to the wilting coefficient.

Points observed in this study which may contribute to the greater drought resistance of shortleaf pine compared to that of loblolly pine are as follows:

1. Shortleaf pine absorbed more water from the soil and at the same time maintained a higher total water content in its leaves even when soil moisture was limited.

2. Shortleaf pine maintained a higher solute concentration when recovering from the effects of drought; that is, when 30 per cent. soil moisture was restored.

The data also show that the greater drought resistance of shortleaf pine cannot be attributed to an ability to conserve water either by retarding transpiration or by forming bound water. Further, the greater drought resistance of this species cannot be attributed to a higher osmotic pressure.

NORTHERN ROCKY MOUNTAIN FOREST  
AND RANGE EXPERIMENT STATION  
MISSOULA, MONTANA

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# SOME INVESTIGATIONS ON THE ASSIMILATION OF APPLE LEAVES

J. G. WAUGH

(WITH FIVE FIGURES)

## Introduction

The assimilation of apple leaves subjected to various environmental conditions has been studied by a number of investigators. Extensive investigations have been made by HEINICKE and HOFFMAN (10) for individual leaves under natural conditions and more recently by HEINICKE and CHILDERS (9) for the foliage of an entire tree. These investigations, however, involved determinations of the average rate of assimilation over periods of several hours. This study was originated to obtain information on the assimilation of individual apple leaves under varying conditions over more brief periods of time than used heretofore. In the following experiments apple leaves were subjected to varying conditions in the laboratory and determinations of the rate of assimilation made in hourly periods.

## Materials and methods

### APPARATUS

MEASUREMENT OF CARBON DIOXIDE.—Assimilation was estimated by determining the differences in carbon dioxide content between a continuous stream of normal air and a similar stream of air that was passed over leaf tissue confined in a special assimilation chamber. The carbon dioxide of the air was determined with a maximum error of 2 per cent. and an average error of approximately 1 per cent. of the atmospheric concentration by an apparatus described by WAUGH (22).

OBTAINING AIR SAMPLES.—Since the experiments were performed in the laboratory where the carbon dioxide content of the air fluctuated violently and was usually considerably higher than that of the normal atmosphere, it seemed desirable to use fresh air from the outside for the experiments. In figure 1 is illustrated the apparatus for obtaining the air samples. *M* represents a leaf on which the experiments are made and *K* an air chamber for drawing air over the leaf. The inlet of the air chamber is fitted with a tube, *T*, which leads to outside air. A suction pump is attached at *J* for drawing air over the leaf and the rate of air flow is determined and adjusted by means of a Sargent wet-test gas meter, *I*, which is accurate to 28 ml. The leaf is subjected to the appropriate external conditions, side tube *E*, on T-tube *G* is shut off, and the three-way stopcock turned so that air is drawn through the air-chamber, *K*, through *L*, through *G* and *H* to the gas meter, *I*, and out at *J*.



The method of taking samples was as follows: A sampling bottle, *A*, was clamped in an inverted position in the ringstand, *F*. The side tube, *E*, was attached to the long tube of the sampling bottle and a small tube, *C*, drawn out to a jet was attached to the short tube of the sampling bottle. This small tube was adjusted to allow the water to run out at an appropriate rate, and consequently determined the rate of flow of air into the sampling bottle. Tubes were made which gave rates of air flow from 5 to 80 liters per hour; where more accurate data were required, stopwatch readings were taken. In

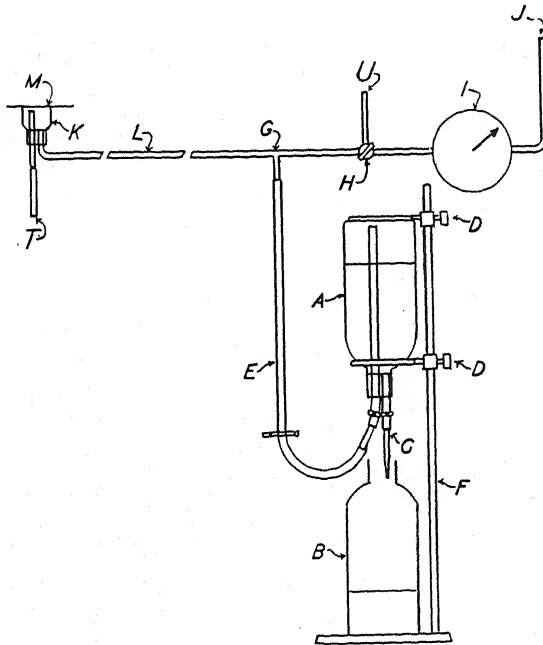


FIG. 1. Method of taking air samples.

some cases when the sample was taken, the suction was shut off from the cup chamber by turning *H*, and the air flowed over the leaf directly to the sampling bottle. In other cases, suction was applied and a sample taken slowly over a long interval of time with a high rate of air flow over the leaf. In fact, a considerable number of variations were obtained and records were kept of the temperature, barometric pressure, and other factors which might have their influence on the data. The method of taking the air check was very simple and consisted in attaching a sampling bottle to a tube which led air in directly from the outside. The carbon dioxide in the air samples was determined by the apparatus described by WAUGH (22).

**AIR CHAMBER.**—In the apple leaf the stomates are confined to the lower side and in figure 2 is given a diagram of an air chamber used in these experi-

ments which is a modification of the original chamber used by HEINICKE (6). The chamber *D* is made by cutting off an 8-oz. cylindrical bottle approximately 6 cm. in diameter about 3 cm. from the shoulder. The cut edge is ground flat and fitted with a rubber cushion *C* covered with soft grafting wax. A 3-hole no. 7 rubber stopper *G* provided with two 6-mm. inside diameter glass tubes, *H* and *I*, and a thermocouple *F* (to be described later), is fitted into the neck of the bottle. A brass ring *A* which holds the leaf *B* against the cushion *C*, is held in position by two rubber bands *EE*. The ring is coated with aluminum paint to reflect light and avoid excessive heating of the metal. It has a felt gasket *J* underneath to prevent injury to the leaf by pressure. The chamber is sturdy and may be clamped rigidly in any suitable position.

Two sizes of air chambers providing effective assimilating leaf areas of 13.18 and 19.64 cm.<sup>2</sup> were used in these experiments. In fitting the leaf to an air chamber, it was carefully pressed down without injuring it by means of the brass ring *A* on the wax-covered rubber cushion, and the wax adapted itself to the veins and contours of the leaf forming a seal which was

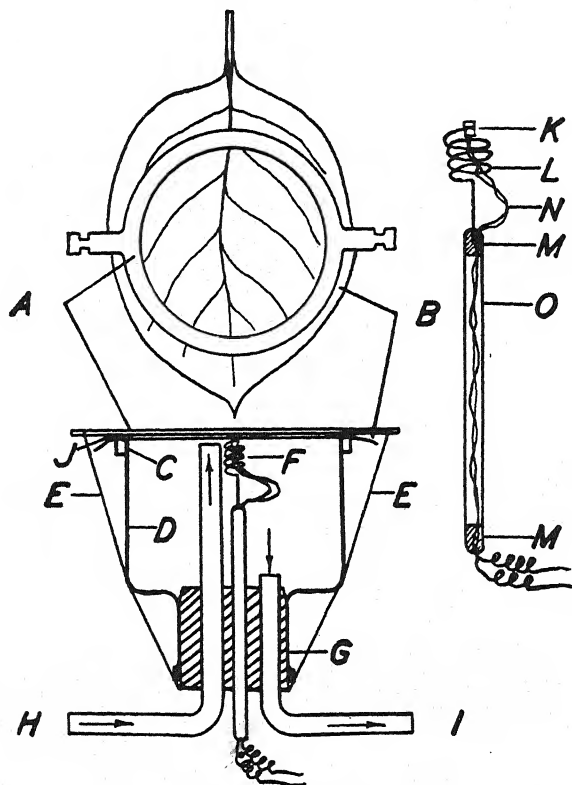


FIG. 2. Air chamber.

very nearly air-tight. Tests indicated that when a healthy apple leaf free from imperfections was used with a rate of air flow of 60 liters per hour through the air chamber, the pressure differential exerted on the leaf was small and the leakage negligible. During the periods when experiments were not being performed, the leaf was removed from the air chamber. The leaf was constantly checked for imperfections and perforations. In re-fitting the leaf to the air chamber, care was taken to make the leaf coincide with the impressions in the wax to obtain a good seal and also that the same portion of the leaf as before came under observation for comparable data.

**MEASUREMENT OF LEAF TEMPERATURES.**—Leaf temperatures were measured with a thermocouple system *F* which is drawn to enlarged scale to the right of the air chamber in figure 2. The thermocouple support consists of a 3-mm. outside diameter glass tube *O* approximately 7.5 cm. long. A light phosphor-bronze wire is shaped into a spiral *L* which holds a small cylinder of cork *K*. On the top of this cork cylinder is supported a copper-constantan thermocouple which lies flat on the surface. The thermocouple leads *N* are brought through the tube *O* and air-tight seals made with sealing wax at *MM*. The thermocouple system was installed in the air chamber so that the thermocouple pressed lightly against the under surface of the leaf near the midrib without injuring it. The thermocouple with its supporting cork cylinder was less than 3 mm. in diameter so that very little of the leaf surface was involved. The reference thermocouple was immersed in the thermostatic water bath of the carbon dioxide measuring apparatus, which was held at  $30^{\circ} \pm 0.01^{\circ}$  C. Leaf temperatures were measured accurate to  $1^{\circ}$  C.

**METHOD OF APPLYING AND MEASURING LIGHT.**—Carbon-dioxide assimilation is expressed on the basis of the milligrams of carbon dioxide absorbed per 100 cm.<sup>2</sup> of leaf surface per hour. Light intensity is expressed in gram calories per cm.<sup>2</sup> of leaf surface per hour, and the milligrams of carbon dioxide absorbed per gram calorie of light may be obtained by the following relation:

$$\frac{\text{mg. CO}_2 \text{ assimilated per gm. cal. of light}}{100 \times \text{gm. cal. light per cm.}^2 \text{ per hour}} = \frac{\text{mg. CO}_2 \text{ assim. per 100 cm.}^2 \text{ per hour}}{100 \times \text{gm. cal. light per cm.}^2 \text{ per hour}}$$

The light intensity may be expressed approximately in foot-candles by the following relationship (12):

$$\text{Light intensity in foot-candles} = 115 \times \text{gm. cal. per cm.}^2 \text{ per hour}$$

A Mazda 150-watt, 120-volt clear glass lamp with 60 cycles A.C. per second was used to supply light to the leaves. In some experiments, it was fitted with a white enamelled reflector. The light intensity applied to the leaf was varied by placing the light at various distances from the leaf and vertical to its surface. An Eppley pyrheliometer (13) was used to determine the light intensity, and calibration curves giving the relation between the dis-

tance of the light from the leaf and the intensity of the light falling on the leaf were constructed. At close range where there was danger of burning the leaf owing to excessive heating, an electric fan was used to cool the leaf. This was placed about 60 cm. from the leaf so that it did not interfere with the light, and the air was driven horizontally across the surface of the leaf.

RECORDS.—Records were kept of the air temperature, leaf temperature, barometric pressure, light intensity, leaf area, rate of air flow, condition of leaf under observation, general condition of the trees and any other factors which might have their influence on the data. Since the temperature of the laboratory was fairly uniform and barometric fluctuations were not excessive, the assimilation and respiration values would not be much affected relatively by converting the carbon dioxide per liter of air to standard conditions of temperature and pressure, or some other standard. Moreover this conversion to some standard would in several respects not be a representative picture of the situation, and assimilation values are given as they were actually obtained.

VARIETIES TESTED.—Experiments were conducted with the leaves of McIntosh, Delicious and seedling apple trees. All the trees were one year old and planted in soil in 5- and 10-gallon galvanized pails. At the time of planting, the nursery stock was cut back to the surface of the soil and only one vigorous shoot allowed to develop from the stub of the scion. The shoots were of varying vigor from 70 to 125 cm. in length and bore leaves ranging from about 45 to 85 cm.<sup>2</sup> in area. The experiments were carried out before and after the terminal bud had formed on the shoots, but in all cases the leaves under experimentation had attained their full size. The trees grew in the greenhouse under favorable conditions until ready for experimentation when they were brought into the laboratory. During the time they were not being used for experiments, they were placed near the laboratory window with the natural light supplemented by the Mazda lamp on dull days. Soil moisture was kept at an optimum since the water content of the leaf has a marked influence on the rate of assimilation (8, 18).

## Experimentation

### SERIES 1

The purpose of these experiments was to ascertain what effect light intensity which was held constant for each experiment, but varied from experiment to experiment, with a slow and constant rate of air flow, and moderate leaf temperatures, had on the hourly course of assimilation and the total assimilation of the leaf.

One leaf was used in these experiments. The rate of air flow through the leaf chamber was 0.38 liters/cm.<sup>2</sup>/hr., and the duration of sampling one-half hour. Different light intensities were used, but the light intensity was held

constant throughout each experiment. With the exception of one or two brief periods when the leaf temperature rose to 36° C. leaf temperatures ranged from 25° to 33° C. One experiment was performed each day and the length of an experiment was 8, 9, or 10 hours. In all, 18 experiments were performed and four examples are shown in figure 4 (A, B, C, D).

TABLE I

ASSIMILATION OF LEAF WITH VARIED LIGHT INTENSITY AND UNIFORM AIR FLOW  
(AIR FLOW 0.38 LITERS/CM.<sup>2</sup>/HR.)

LIGHT INTENSITY	DURATION OF EXPERIMENT	TOTAL ASSIMILATION	ASSIMILATION ON ONE-HOUR BASIS	AVERAGE ASSIMILATION ON ONE-HOUR BASIS	CO <sub>2</sub> ASSIMILATED PER GM. CAL. LIGHT × 10 <sup>4</sup>
$\frac{\text{gm. cal.}}{\text{cm.}^2/\text{hr.}}$	hr.	$\frac{\text{mg. CO}_2}{100 \text{ cm.}^2}$	$\frac{\text{mg. CO}_2}{100 \text{ cm.}^2/\text{hr.}}$	$\frac{\text{mg. CO}_2}{100 \text{ cm.}^2/\text{hr.}}$	mg.
5.4	10	19.6	1.96	1.75	32.4
	10	16.9	1.69		
	10	14.4	1.44		
	10	19.2	1.92		
10.8	9	19.5	2.17	2.55	23.6
	10	23.0	2.30		
	9	26.6	2.96		
	10	23.8	2.38		
	9	21.9	2.43		
	10	30.8	3.08		
26.4	8	30.5	3.82	3.34	12.7
	9	26.1	2.90		
	10	32.9	3.29		
38.4	10	40.6	4.06	4.03	10.5
	10	33.9	3.39		
	10	46.4	4.64		
97.8	10	35.7	3.57	4.14	4.2
	10	47.0	4.70		

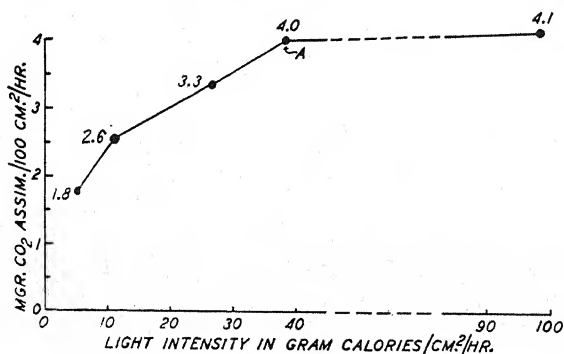


FIG. 3. Effect of light intensity on assimilation with limiting air supply.

In table I are given the experiments performed, their duration and total assimilations. For purposes of comparison, since the experiments are not all of the same length, the average assimilation on an hour basis has been obtained by dividing the total assimilation by the duration of the experiment. The results are shown graphically in figure 3.

### SERIES 2

The purpose of these experiments was to ascertain the influence of two rates of air flow on the assimilation of a leaf with constant light intensity and moderate leaf temperatures.

In these experiments one leaf was used. The rates of air flow were 0.38 liters/cm.<sup>2</sup>/hr. and 1.9 liters/cm.<sup>2</sup>/hr. Air samples were taken at the beginning of each hour interval. The time of sampling with the higher rate of air flow was 6 min.; with the lower rate, 30 min. Leaf temperatures ranged mostly between 24° and 33° C. One experiment was performed each day and the duration of each experiment was 10 hours. In all, 14 experiments were performed and two examples of the data for the higher rate of air flow are given in figure 4 (E, F).

The total assimilation obtained for each experiment was divided by 10 to obtain the average rate of assimilation per hour and these values for various light intensities and rates of air flow are given in columns 2 and 4 of table II. In columns 3 and 5 are given the averages of the assimilation per hour, and in columns 6 and 7 are given the average milligrams of carbon dioxide assimilated per gram calorie of light received.

TABLE II  
ASSIMILATION OF LEAF WITH VARIED LIGHT INTENSITY AND TWO DIFFERENT  
RATES OF AIR FLOW

LIGHT INTENSITY (GM. CAL./ CM. <sup>2</sup> HR.)	ASSIMILATION ON ONE-HOUR BASIS (MG. CO <sub>2</sub> /100 CM. <sup>2</sup> /HR.)				AVERAGE MG. OF CO <sub>2</sub> ASSIMILATED PER GM. CAL. OF LIGHT			
	AIR-FLOW (0.38 LITERS/CM. <sup>2</sup> /HR.)		AIR-FLOW (1.9 LITERS/CM. <sup>2</sup> /HR.)		AIR-FLOW (0.38 LITERS/CM. <sup>2</sup> /HR.) × 10 <sup>4</sup>		AIR-FLOW (1.9 LITERS/CM. <sup>2</sup> /HR.) × 10 <sup>4</sup>	
<i>gm. cal.</i>	<i>mg.</i>	<i>Average mg.</i>	<i>mg.</i>	<i>Average mg.</i>	<i>mg.</i>	<i>%</i>	<i>mg.</i>	<i>%</i>
12.0	1.29		6.47		11.4	100	48.5	100
	1.44	1.37	5.17	5.82				
20.4	0.74		8.25		7.2	63	44.7	92
	1.80		9.97	9.11				
	2.10							
	1.24	1.47						
37.2	2.46		11.14		6.0	53	35.0	72
	1.98	2.22	14.87	13.01				

## SERIES 3

The purpose of these experiments was to ascertain the influence of light intensity varied hourly during the course of the experiment on the assimilation of the leaf with a constant rate of air flow and moderate leaf temperatures.

One leaf was used in these experiments. The rate of air flow was 1.9 liters/cm.<sup>2</sup>/hr. and leaf temperatures ranged from 26° to 32° C. Changes in light intensity were made at the beginning of the hour interval and the air samples were taken at the middle of the hour interval. This was done in order to allow the leaf time to adjust its metabolic processes to the changed light intensity. In all experiments, the light intensity was progressively increased to a maximum and then progressively decreased to a minimum. The same sequence of light intensities was used in all experiments. Eight experiments of eight hours duration were performed and the results are given in table III. Two examples are shown in figure 4 (G, H).

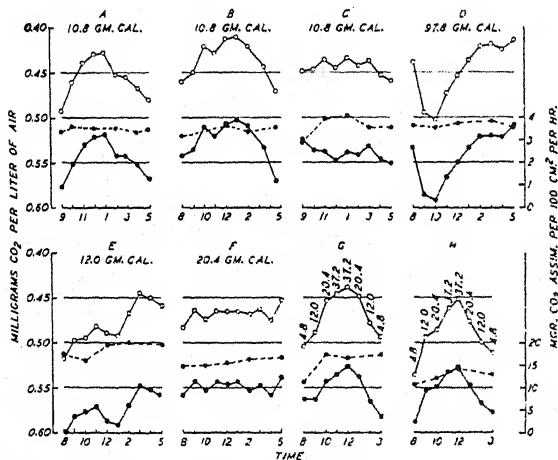


FIG. 4. Effect of various external conditions on assimilation. Solid line with open circles: carbon dioxide content of air passed over leaf (left ordinate); broken line with solid circles: carbon dioxide content of air check (left ordinate); solid line with solid circles: carbon dioxide assimilated per 100 square centimeters of leaf surface per hour (right ordinate). Light intensities given in gram calories per cm.<sup>2</sup> per hour.

## SERIES 4

The purpose of these experiments was to ascertain the influence of leaf temperature varied hourly during the course of the experiment on assimilation with constant light intensity and rate of air flow.

One leaf was used in these experiments. The leaf temperature was varied by the electric fan, and changes in leaf temperatures were made at the be-

TABLE III

VARIAION IN ASSIMILATION WITH HOURLY VARIATION IN INTENSITY OF ILLUMINATION  
(AIR FLOW 1.9 LITERS/CM.<sup>2</sup>/HR.)

EXPERIMENT	LIGHT INTENSITY (GM. CAL./CM. <sup>2</sup> /HR.)							
	4.8	12.0	20.4	37.2	37.2	20.4	12.0	4.8
	ASSIMILATION (MG. CO <sub>2</sub> /100 CM. <sup>2</sup> /HR.)							
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
1	5.9	8.4	10.1	11.6	13.5	11.7	7.7	5.1
2	7.5	7.6	11.2	13.0	14.8	11.0	7.2	3.6
3	2.0	9.2	10.3	13.5	14.6	10.3	6.7	4.8
4	0.5	0.2	7.5	15.2	10.3	8.9	12.5	3.8
5	5.5	4.6	6.4	14.0	15.6	15.1	7.6	7.7
6	3.0	7.2	8.5	20.2	16.5	13.4	9.1	8.4
7	3.6	6.8	9.3	13.0	12.0	8.6	4.9	2.7
8	2.8	9.5	7.3	17.9	15.8	12.7	8.1	3.1
Average assimilation	3.9	6.7	8.8	14.8	14.1	11.5	8.0	4.9
Average mg. CO <sub>2</sub> assimilated per gm. cal. of light × 10 <sup>4</sup>								
	81.3	55.8	43.1	39.8	37.9	56.4	66.7	102.1
	80%	55%	42%	39%	37%	55%	65%	100%

ginning of the hour intervals. Air samples were taken at the middle of the intervals to allow the leaf to adjust its metabolic processes to the changed leaf temperature. The rate of air flow was 1.9 liters/cm.<sup>2</sup>/hr. Six experiments, using a temperature range of approximately 30° to 38° C., were performed and two examples are given in figure 5.

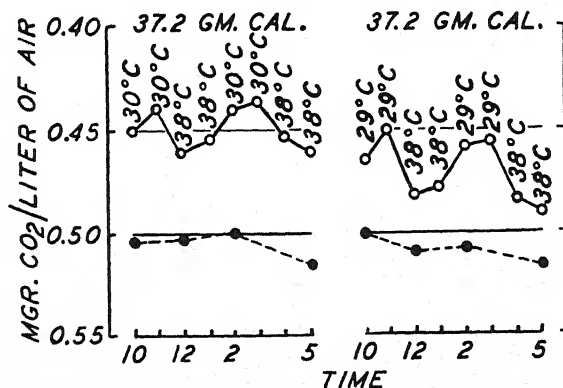


FIG. 5. Effect of leaf temperature on assimilation. Solid line with open circles: carbon dioxide content of air passed over leaf; broken line with solid circles: carbon dioxide content of air checks. Light intensities given in gram calories per cm.<sup>2</sup> per hour.



### Discussion

From series 1 and 2, figure 4 (A to F), it is seen that the assimilation of the apple leaf with uniform light intensity, restricted leaf temperature variation, adequate water supply, and a slow constant rate of air flow was very irregular from hour to hour on some days and fairly constant on others. In general the assimilation starts out relatively low and increases with one, two, or several maxima during the experiment. In some experiments the assimilation subsides and reaches a relatively low value again toward the close of the experiment.

It would be difficult to designate specific external factors that might operate to cause such wide fluctuations in assimilation under these fairly uniform external conditions. Although the lamp used to illuminate the leaf was supplied with 60 cycles A.C. per second, it seems unlikely that the resultant fluctuating illumination would cause any more appreciable difference in the response of the leaf than if it were under steady illumination. McALISTER (19) has shown that with high frequencies such as 1/60-second periods of light and darkness, the induction period is very small, and the assimilation practically the same as under steady illumination.

While fluctuations in the carbon dioxide content of the air would be expected to influence assimilation, the absence of correlation between the fluctuations in assimilation and carbon dioxide content of the atmosphere indicates that other factors predominate. This is also indicated by the results of series 3, table III, where assimilations obtained with the same light intensity and external conditions show considerable variation within an experiment and from day to day. HEINICKE and HOFFMAN (10) found that with individual apple leaves there were violent fluctuations in assimilation from day to day which could not always be correlated with prevailing external conditions. They state: "On the whole, the internal conditions which govern the supply of water and nutrients and the translocation and utilization of assimilated materials seem to have a profound influence on the efficiency of the foliage of the apple as well as that of other plants." [See also HEINICKE (7), HEINICKE and CHILDERS (8), CHILDERS and COWART (3).] HEINICKE and CHILDERS (9), working with the foliage of an entire tree, conclude that while variations in carbon dioxide concentration may have some influence in determining the rate of apparent photosynthesis, other factors are more important under natural conditions. KOSTYTSCHEW and his co-workers (14, 15, 16, 17) have pointed out that the directly limiting factors are probably internal and such factors are subject to varying degrees of stimulation by light and temperature, and so on, depending on the time of the day or the condition of the tissue. [See also BOONSTRA (2), TSESCHNOKOV and BAZYRINA (21).] HARDER (5) and ARNOLD (1) have shown that in some plants assimilation will fall off as a result of fatigue even though light, temperature, and carbon dioxide of the air remain constant.

From series 2, table I and figure 3, there is a very apparent increase of the average rate of assimilation per hour with increase in light intensity up to 38.4 gm.cal./cm.<sup>2</sup>/hr. (fig. 3, A), but for a light intensity of 97.8 gm.cal./cm.<sup>2</sup>/hr., there is very little further increase. It seems likely that the rate of air flow, 0.38 liter/cm.<sup>2</sup>/hr., becomes limiting for this high light intensity. HEINICKE and HOFFMAN (10) have shown that the maximum rate of assimilation of apple leaves on clear days (where the light intensities were probably not greater than 97.8 gm.cal./cm.<sup>2</sup>/hr.) is considerably limited by rates of air supply less than 1 liter/cm.<sup>2</sup>/hr., and CHRISTOPHER (4) also working with apple leaves has found that an air supply has a marked limiting influence on assimilation especially where low air supplies in the neighborhood of 1 liter/cm.<sup>2</sup>/hr., or less, are used. In table I it is seen that the average milligrams of carbon dioxide assimilated per gm. cal. of light decrease as the light intensity increases. From the rapidity with which these values decrease, it seems probable that the air flow of 0.38 liter/cm.<sup>2</sup>/hr. becomes appreciably limiting even at the lower light intensities.

The results of series 2, figure 4 (E, F), show irregular fluctuations in assimilation as in series 1, figure 4, (A to D). Calculation of the ratios between average hourly assimilations at corresponding light intensities in table II gives the values 4.3, 6.2, and 5.9. Roughly the ratio is in the region of 5, the ratio of the two rates of air flow. The results illustrate the importance of an adequate air supply to the leaf and indicate that the air flow of 0.38 liter/cm.<sup>2</sup>/hr. was limiting. It is likely that the air flow of 1.9 liters/cm.<sup>2</sup>/hr. was also limiting. This conclusion is supported by the results of HEINICKE and HOFFMAN (10) and CHRISTOPHER (4), which indicate that an air supply of at least 2.5 liters/cm.<sup>2</sup>/hr. is necessary to simulate natural conditions in photosynthetic studies. From table II, columns 6 and 8, it is seen that the mg. of carbon dioxide assimilated per gm. cal. of light decrease for both rates of air flow as the light intensity is increased. If, however, 100 per cent. is arbitrarily assigned to the values obtained for the light intensity of 12.0 gm.cal./100 cm.<sup>2</sup>/hr. and the other values expressed as percentages of these values, it is seen that the percentages decrease more rapidly for the lower rate of air flow. Evidently the limiting action of the lower rate of air flow becomes increasingly greater over the higher rate of air flow as the light intensity is increased.

From the above results it is apparent that the ranges of air supply and light intensity used in this study are limiting factors. HOOVER, JOHNSTON, and BRACKETT (11), working with wheat under carefully controlled external conditions, observed that there is a limited transition region over which both the carbon dioxide concentration and light intensity seem to be limiting factors. While they kept their rate of air flow to the plant constant, and varied the carbon dioxide concentration of the air instead of the reverse as was done

in the above experiments, both methods are means of carbon dioxide supply and the results seem to be comparable. They ascribe this transition region to a lack of homogeneity of light intensity and carbon dioxide concentration throughout the leaf which may explain, in part, the results above.

The response of assimilation to changes in light intensity is shown in series 3, figure 4 (G, H), and table III. The averages of the assimilations obtained for each light intensity, taken in the order in which they were used in the experiments, indicate that in general there is a response in assimilation to changes in light intensity, and that this adjustment of assimilation to light intensity takes place within an hour interval.

There is also fair agreement in average assimilations obtained for corresponding light intensities and rates of air flow in tables II and III. A close relationship between photosynthesis and illumination in hour intervals has been observed by THOMAS and HILL (20) for wheat and alfalfa. Responses in the assimilation of apple leaves to changes in light intensity over longer periods of time have been observed by HEINICKE and HOFFMAN (10) for individual leaves and HEINICKE and CHILDERS (9) for tree foliage. McALISTER (19), working with wheat under carefully controlled external conditions, has obtained short induction periods of a few minutes for illumination, furnishing additional evidence of the rapidity of response of the assimilatory mechanism to changes in light intensity.

Series 4, figure 5, indicates that high leaf temperatures such as 37° or 38° C. result in a reduced assimilation of the leaf as compared with temperatures around 30° C. For individual apple leaves HEINICKE and HOFFMAN (10) find that higher temperatures may, under certain conditions, reduce the rate of apparent photosynthesis. They find that the critical temperature of the apple leaf is about 35° C. which appears to be in accord with the above data. For apple foliage HEINICKE and CHILDERS (9) find that high temperatures cause a reduction in assimilation. They conclude that the rate of respiration is influenced more by temperature than is the rate of photosynthesis, the higher temperatures favoring higher respiration with a reduction in the rate of apparent photosynthesis. They also advance the possibility of higher temperatures interfering with the photosynthetic mechanism and directly reducing assimilation. THOMAS and HILL (20) note that at times the photosynthetic maximum is appreciably higher than normal when the sun is intermittently obscured by clouds for a few seconds to one or two minutes. They attribute it in part to a lower leaf temperature with attendant lower respiration and possibly in part to the orientation of the chloroplasts. McALISTER (19) obtained progressive increases in respiration with temperatures of 12°, 21°, and 31° C., and found that respiration was independent of light intensity. From the apparatus and technique employed it seems very likely that the respiration values he obtained represent the respiration actually tak-

ing place during photosynthesis. It seems likely in the experiments reported here that the reduction in apparent assimilation at the higher temperatures was in part, at least, caused by increased respiration with possible inhibition of the photosynthetic mechanism.

### Summary

1. Apple leaves were subjected to various external conditions in the laboratory and determinations of the rate of assimilation made in hour periods.

2. The results indicate that under fairly uniform external conditions, the rate of assimilation of apple leaves is irregular. On some days the rate of assimilation is fairly uniform and on other days it fluctuates appreciably from hour to hour and may have several maxima in the course of a day. It seems probable that internal factors play a significant and important part in the assimilation of the apple leaf.

3. The results indicate that under fairly uniform external conditions the response in assimilation of the apple leaf to changes in light intensity take place within an hour.

4. Rates of air flow of 0.38 and 1.9 liters per cm.<sup>2</sup> of leaf surface per hour used with low and moderate light intensities gave rates of assimilation for the same light intensities that were roughly in proportion to the ratio of the rates of air flow. The limiting action of the lower rate of air flow became increasingly greater over the higher rate of air flow as the light intensity was increased. Further studies indicated that the lower rate of air flow became almost completely limiting at high light intensity.

5. The results obtained with the rates of air flow and light intensities used in these studies indicate that in the assimilation of the apple leaf there is a transition range over which light intensity and rate of air flow are both limiting factors.

6. In general, under fairly uniform external conditions, leaf temperatures of about 38° C. seemed to result in reduced assimilation as compared to leaf temperatures of about 30° C. It is probable that this reduction in assimilation at higher leaf temperatures was due in part, at least, to increased respiration with possible inhibition of the photosynthetic mechanism. The results seem to support the conclusion of other investigators that the critical temperature of the apple leaf is about 35° C.

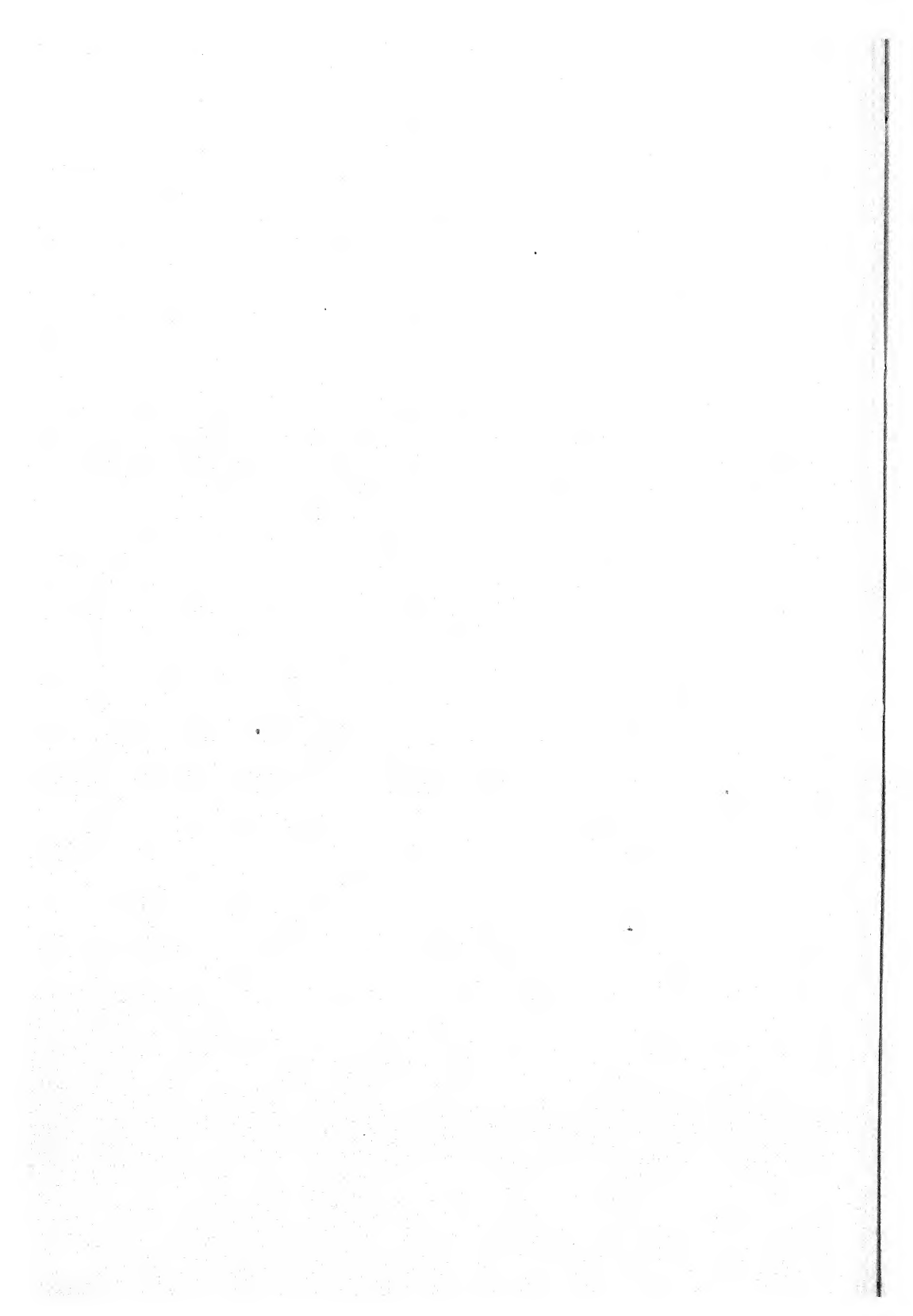
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# INFLUENCE OF CERTAIN AMINO ACIDS AND OF NICOTINIC ACID UPON THE NICOTINE CONTENT OF TOBACCO LEAVES<sup>1</sup>

RAY F. DAWSON

## Introduction

The tobacco alkaloid nicotine possesses a molecular structure by which it may be related empirically to the amino acid proline. Proline is  $\alpha$ -pyrrolidinecarboxylic acid. Nicotine may be considered as an N-methylated proline containing a  $\beta$ -pyridine ring attached to the alpha position in place of the carboxyl. These similarities have been pointed out by TOTTINGHAM (9) and have been employed by TRIER (12) as a basis for extensive speculations on the probable origin of nicotine in the tobacco plant. According to TRIER, nicotine may be formed *in vivo* from proline and nicotinic acid by a simultaneous oxidative decarboxylation of the two molecules. The methylation of the pyrrolidine nitrogen was accounted for by the action of methyl alcohol as a dismutation product of formaldehyde. TRIER postulated the formation of the pyridine portion of the nicotine molecule from proline through the intervention of formic acid as the second dismutation product of formaldehyde. In short, the hypothesis of TRIER assigned to proline the rôle of precursor of both the five- and the six-membered rings of the nicotine molecule. It should be noted, however, that TRIER's theory was advanced at a time when nicotinic acid was practically unknown in the literature of plant chemistry.

KLEIN and LINSER (7) tested TRIER's hypothesis by culturing the excised leafy shoots of tobacco plants in dilute aqueous solutions of proline. These investigators obtained large increases in the nicotine content of the leaves and stems of the plants to which proline had been supplied. The hydrochlorides of glutamic acid and of ornithine were found to possess similar effects. GORTER (5) critically reviewed the work of KLEIN and LINSER and presented data of his own to show that both those plants provided with proline and those cultured in KNOP's solution after a period of 4 or 5 days contained less nicotine than at the beginning of the experiment. The increase in nicotine content of the leaves of the plants supplied with proline was only apparent and represented merely a less extensive decrease rather than an actual synthesis of nicotine. In the present study attention was given to the influences of proline, pyrrolidonecarboxylic acid, glutamic acid, ornithine, nicotinic acid, and other substances upon the nicotine content of tobacco leaves by the use of methods of culture and of expression of data

<sup>1</sup> Contribution from the Osborn Botanical Laboratory, Yale University.



differing in certain respects from those employed by KLEIN and LINER and by GORTER.

### Materials and methods

Tobacco seedlings of the Havana Seed and Connecticut Broadleaf no. 38 varieties were grown in white quartz sand to which a modified SHIVE three-salt nutrient solution was added at daily intervals. The composition of this solution was calcium nitrate 0.0042, monopotassium phosphate 0.0021, and magnesium sulphate 0.0042 partial gram moles per liter. Supplements of iron, boron, manganese, and copper were added in the proportions of two parts per million. The seedlings were transplanted when about one inch in height to a well composted soil in eight-inch porous clay pots. Weekly applications of a 0.1 per cent. suspension of Nitrophoska (15-30-15), extension of the daily photoperiod by a series of 150-watt Mazda lamps, and an average greenhouse temperature of 25° C. made possible the production of fairly vigorous plants with large dark green leaves even during the winter season. In all cases the plants were grown in the greenhouse between the months of September and June.

When the plants possessed 10 to 15 large leaves each, they were carefully selected for uniformity in height of stem and leaf development. In general, from 4 to 6 plants were used in each experimental unit. An initial leaf sample and a final leaf sample taken from normal rooted plants at the beginning and the end of each experiment provided supplementary controls and served to indicate the course of nicotine synthesis under normal conditions of plant growth.

At the end of the experimental period the leaves were taken from the stems and placed in a refrigerator to await preparation for drying. The midribs were removed and the leaf blades immediately dried in a forced current of air preheated to 80° C. Drying was usually complete within an hour, but the tissues were allowed to remain in the oven for 2 hours. The material was then pulverized, weighed, and preserved in tightly stoppered bottles. The results of subsequent chemical analyses are expressed on the basis of this roughly dried material.

The cut stem method of plant culture as employed by KLEIN and LINER (7) in their studies on nicotine metabolism was adapted for large scale use in the present investigation. The details of this modified procedure have been described elsewhere (3). Essentially, the technique involves the use of low temperatures to prevent decomposition of the culture solutions by microorganisms. The necessity of sterilizing plant tissues by chemical means and of autoclaving heat unstable organic compounds was thus avoided. The culture solutions were made up by dissolving the desired quantities of material in tap water and adjusting the acidity to about pH 3.0.

Nicotine was determined by steam distillation from alkaline solution (1). Duplicate or triplicate determinations were made for each sample of tissue. Approximately 2 grams of dried leaf were used for each distillation, and 750 ml. of distillate were collected from each sample.

All data from these experiments are expressed in terms of milligrams of nicotine in the leaves of the individual plant. All changes in volatile alkaloid content amounting to less than 20 per cent. are arbitrarily considered to be within the limits of experimental error and hence of no value in establishing the premises of this study.

Glutamic acid was prepared by the addition of one equivalent of potassium hydroxide to a solution of *d*-glutamic acid hydrochloride. The precipitate was filtered, washed with water, and dried. Pyrrolidonecarboxylic acid was obtained by autoclaving a 10-per cent. suspension of glutamic acid prepared as previously described at 115 pounds pressure for 6 hours (11). Since no ready method was available for the determination of the components of the resulting equilibrium mixture, it was assumed for purposes of making up the culture solutions that the reaction had proceeded to completion. All the amino acids, sugars, and other compounds and reagents used in this study beyond those whose preparations have already been described were the highest grades obtainable from Merck and Co., Inc., and from the Eastman Kodak Co.

Nicotine was isolated as the dipicrate from the steam distillate of each sample showing a significant deviation from the controls with respect to nicotine content. The purpose of this operation was to determine from the melting point of each preparation whether or not the increase in nicotine content in the tissues as determined by the steam distillation method represented an actual synthesis of nicotine itself or merely a conversion of the substances supplied in the culture solutions into steam volatile bases which could be precipitated with silicotungstic acid and, hence, be determined as nicotine. The possibility of such interference seemed especially likely in the case of proline and nicotinic acid, both of which could conceivably be decarboxylated within the leaf tissues to yield pyrrolidine and pyridine respectively. The melting point of once-recrystallized nicotine dipicrate isolated from a sample of normal leaf tissue was 221–222° C. (uncorrected).

## Results

### THE EFFECTS OF GLUTAMIC ACID AND OF PYRROLIDONECARBOXYLIC ACID UPON THE NICOTINE CONTENT OF TOBACCO LEAVES

TRIER (12) related glutamic acid and its lactam pyrrolidonecarboxylic acid to nicotine synthesis through proline. Theoretically, it is possible that glutamic acid may give rise to proline in plant tissues by the reactions glutamic acid  $\rightarrow$  pyrrolidonecarboxylic acid  $\rightarrow$  proline. The first step is easily

carried out in the laboratory by heating an aqueous solution of glutamic acid as has been described. The reduction of the carbonyl oxygen of pyrrolidonecarboxylic acid to the  $\text{CH}_2$  of pyrrolidinecarboxylic acid (proline) is much more difficult to accomplish (4). In the present study two experiments were conducted employing both glutamic acid and pyrrolidonecarboxylic acid in the culture solutions.

In the first experiment, 24 Connecticut Broadleaf no. 38 tobacco plants were placed, in the manner already described for cut stem cultures, in 0.007 M solutions of *d*-glutamic acid, *l*-pyrrolidonecarboxylic acid, and citric acid all adjusted to pH 3.4. The flasks containing the leafy shoots were placed in a diffusely lighted room the relative humidity of which remained about 35 per cent. The solutions were maintained at a temperature of about 10° C. After 96 hours of culture the plants were harvested and dried. The quantities of solution absorbed, the dry weights, and the data for nicotine content are found in table I.

TABLE I

EFFECTS OF *d*-GLUTAMIC ACID, *l*-PYRROLIDONECARBOXYLIC ACID, AND OF CITRIC ACID ON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 96 HOURS.  
LEAVES HARVESTED MARCH 27, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	<i>gm.</i>	<i>%</i>	<i>mg.</i>	<i>%</i>	<i>ml.</i>
Initial controls .....	4.9	1.62	79	+ 10	
Water .....	4.9	1.47	72	0	270
Pyrrolidonecarboxylic acid .....	5.0	1.80*	90	+ 25	313
Glutamic acid .....	5.3	1.40	74	+ 3	465
Citric acid .....	4.8	1.43	69	- 4	321
Final controls (rooted plants)...	5.4	1.50	81	+ 13	

\* Once-recrystallized picrates from the steam distillate melted at 221-222° C. (uncorrected).

The results indicate an increase in absolute nicotine content only in the leaves of those plants which had been supplied with pyrrolidonecarboxylic acid. This increase amounted to nearly 25 per cent. of the quantity of volatile alkaloid present in the leaves of the control plants in water. Glutamic acid exerted no influence on nicotine formation, but there were unmistakable indications of its effects on solution absorption and leaf turgidity (3). Citric acid also failed to alter the process of nicotine synthesis.

A second experiment employing *d*-glutamic acid and *l*-pyrrolidonecarboxylic acid was conducted under the same conditions as were described for the preceding experiment. The plants used were similar with respect to

size and stage of development, and the culture solutions were the same except that glycine was substituted for citric acid and that all the solutions were adjusted to pH 3.0. Each experimental unit consisted of 5 Connecticut Broadleaf no. 38 tobacco plants. After 100 hours of culture the plants were harvested and the leaves dried. The dry weights and analytical data are presented in table II A.

TABLE II

EFFECTS OF *D*-GLUTAMIC ACID, *L*-PYRROLIDONECARBOXYLIC ACID, AND OF GLYCINE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	gm.	%	mg.	%	ml.
(A) Culture period 96 hours. Leaves harvested April, 6, 1937					
Initial controls .....	6.1	1.50	92	+ 2	
Water .....	6.1	1.47	90	0	330
Pyrrolidonecarboxylic acid .....	6.6	1.84	121*	+ 35	346
Glutamic acid .....	6.4	1.54	99	+ 10	432
Glycine .....	6.0	1.72	103	+ 14	362
Final controls (rooted plants)...	6.3	1.57	99	+ 10	
(B) Culture period 40 hours. Leaves harvested April 17, 1937					
Water .....	6.46	1.11	72	0	210
Glutamic acid .....	6.39	1.45	93	+ 29	396

\* Once-recrystallized picrates from the steam distillate melted at 221-222° C. (uncorrected).

Again pyrrolidonecarboxylic acid gave a significant increase in absolute nicotine content: this time the increase was 35 per cent. above the corresponding value for the leaves of the plants in water. Glutamic acid, however, failed to influence nicotine formation in spite of the marked physiological influence which it again exhibited upon the plants to which it was supplied. Glycine gave an apparent increase of 14 per cent. in nicotine. The order of magnitude of this variation, however, lies well within the limits of experimental error and cannot be regarded as significant.

The evidence of physiological activity exhibited by glutamic acid in the two experiments just described led to the suggestion that the quantity of the acid supplied to the plants may have been inadequate for nicotine formation from the acid as a precursor because of its rapid utilization in other cell reactions of considerably greater intensity. Consequently, an experiment was conducted in which twice the concentration of glutamic acid was em-

ployed in the culture solutions. Ten plants (Connecticut no. 38) were placed with their cut stems in 0.014 M solutions of *d*-glutamic acid and in water, both adjusted to pH 3.2. Room temperature remained around 22° C., and the light was diffuse. At the end of 40 hours all the plants were harvested and the leaves dried.

Apparently in close agreement with the suggestions advanced above, the analytical results (table II B) indicate a synthesis of alkaloid in those leaves supplied with glutamic acid to the extent of 29 per cent. above the amount present in the leaves of the plants in water. Such an increase, although apparently well above the limits of arbitrarily established experimental error, is considered insufficient alone to establish a relationship between this amino acid and the process of nicotine synthesis. Indeed, glutamic acid is sufficiently far-removed from the end product of TRIER's scheme that no very decisive or consistent results might be expected from its use in experiments of this type.

The results for the influence of pyrrolidonecarboxylic acid upon nicotine synthesis, however, seem to be much more suggestive. From the evidence at hand and that of KLEIN and LINSER (6) upon the synthesis of trigonelline from pyrrolidonecarboxylic acid, it appears that some mechanism may be available in the leaf cells by which this substance can be converted into nicotine or related compounds.

#### THE EFFECT OF PROLINE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES

The third step in the hypothetical nicotine synthesis of TRIER (12) involves the simultaneous oxidative decarboxylation of a molecule of *n*-methyl proline and a molecule of nicotinic acid with subsequent combination of the cyclic residues to give the characteristic structure of the nicotine molecule. It should be noted that the substance which would be formed in this reaction from proline itself with nicotinic acid is identical with one of the minor tobacco alkaloids closely associated with nicotine, namely nornicotine (8). KLEIN and LINSER (7) reported the results of eight different experiments in which proline alone gave an average increase of about 80 per cent. in the nicotine content of the green tobacco plants. Their conclusion agrees in principle with the hypothesis of TRIER that proline acts as the precursor not only of the pyrrolidine but also of the pyridine ring structures of the nicotine molecule.

In the first attempt to repeat the work of these authors, 24 Connecticut no. 38 tobacco plants were placed under conditions of cut stem culture in 0.1 per cent. solutions of *d*-arginine monohydrochloride, *L*-proline, *DL*- $\alpha$ -amino-*n*-valeric acid, nicotinic acid hydrochloride, and sodium magnesium

TABLE III

EFFECTS OF *L*-PROLINE AND OF OTHER SUBSTANCES UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 96 HOURS.

LEAVES HARVESTED JUNE 14, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	<i>gm.</i>	<i>%</i>	<i>mg.</i>	<i>%</i>	<i>ml.</i>
Initial controls .....	9.2	1.15	106	+ 13	
Water .....	7.1	1.33	94	0	190
Sodium magnesium chlorophyllin .....					
$\alpha$ -Amino- <i>n</i> -valeric acid .....	7.4	1.33	98	+ 4	137
Arginine monohydrochloride .....	7.1	1.38	98	+ 4	150
Nicotinic acid hydrochloride .....	8.0	1.54	123*	+ 31	500
Proline .....	7.4	1.56	115†	+ 22	200
Final controls (rooted plants)...	11.5	1.06	121	+ 29	

\* Once-recrystallized picrates from the steam distillate melted at 221–222° C. (uncorrected).

† Once-recrystallized picrates from the steam distillate melted at 220–221° C. (uncorrected).

chlorophyllin<sup>2</sup> and in water. The solutions were chilled to 16° C. by use of running water. The light was diffuse and indirect, and the room temperature remained between 22° and 23° C. The leaves were harvested and dried at the end of the fourth day, at which time only the plants supplied with nicotinic acid hydrochloride were still fresh and turgid. The plants which had been placed in solutions of sodium magnesium chlorophyllin wilted within 2 hours after the inception of the experiment and were hence discarded.

Two of the substances employed in the culture solutions in this experiment induced significant variations in the nicotine content of the leaves. *L*-Proline apparently increased the absolute nicotine content of the leaves 22 per cent. above the quantity of the alkaloid present in those leaves cultured in water. Nicotinic acid hydrochloride exerted a similar influence (table III). The magnitude of this figure for the effects of proline on nicotine content lies very close to the limits of experimental error. A second experiment was conducted, therefore, to serve as a check on the preceding one.

Twenty Connecticut no. 38 plants were placed with their stems in 0.014 M solutions of glucose, glucose plus ammonium chloride, and glycine, and in

<sup>2</sup> Sodium magnesium chlorophyllin was supplied through the courtesy and kindness of Dr. F. M. SCHERTZ of the American Chlorophyll Co., Alexandria, Va.

0.1 per cent. solutions of *l*-proline and *d*-arginine monohydrochloride at pH 3.0. These plants were in the period of most rapid growth during the month of May, and, hence, probably represented the nearest approach to carbohydrate-high summer plants of any used in this study. Throughout the experiment the room temperature fluctuated between 20° and 25° C., while the relative humidity remained around 67 per cent. Light was indirect and diffuse. After 70 hours the leaves were harvested and dried.

An unmistakable increase of 50 per cent. in the total nicotine content of the leaves followed the absorption of proline. Glycine, glucose, glucose plus ammonium chloride, and arginine, respectively, failed to exert a significant influence on nicotine synthesis or utilization under the conditions of this experiment. The order of magnitude of the increase induced by proline in this instance seems to lie well outside the limits of experimental error and likewise seems to provide a very good check against the results obtained in the preceding experiment. The data for this experiment are found in table IV.

TABLE IV

EFFECTS OF *l*-PROLINE AND OF OTHER SUBSTANCES UPON THE NICOTINE CONTENT OF TOBACCO LEAVES AFTER CUT STEM CULTURE FOR 69 HOURS.  
LEAVES HARVESTED JUNE 13, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	<i>gm.</i>	%	<i>mg.</i>	%	<i>ml.</i>
Initial controls .....	5.16	1.00	62	+ 4	
Glucose .....	4.50	1.11	50	0	430
Glucose plus ammonium chloride...	4.56	1.16	53	+ 6	430
Glycine .....	4.26	1.06	45	- 10	485
<i>d</i> -Arginine monohydrochloride .....	4.50	1.08	49	- 2	267
<i>l</i> -Proline .....	4.98	1.50	75*	+ 50	233
Final controls (rooted plants)...	5.88	1.11	65	+ 30	

\* Once-recrystallized picrates from the steam distillate melted at 221-222° C. (uncorrected).

Taken as a whole, the data from this study and from that of KLEIN and LINSEER seem to establish the fact that tobacco leaf tissues have the capacity for transforming the pyrrolidine nucleus of proline into nicotine. Inspection of the melting point values for the picrates isolated from the distillation mixtures indicates that they were identical with the melting point of nearly pure nicotine dipicrate. Hence, although it is barely possible that picrates of such other substances as pyrrolidine or pyridine might have escaped detection by means of greater solubility in the aqueous recrystal-

lizing medium, it seems reasonable to assume that the differences in volatile alkaloid content of the leaf samples employed in this study might be safely attributed to increased synthesis of nicotine itself.

One other important aspect of the data should be considered. The work of GORTER (5) appears to show that increases in nicotine content obtained by the use of proline were caused by a less extensive decrease in nicotine content rather than by an actual increase of alkaloid in the plant tissues. This investigator reported losses during water culture in the nicotine content per unit leaf area of an order of magnitude considerably in excess of the values obtained in the extensive investigations of VICKERY *et al.* (10). In the present study small losses of volatile alkaloid have been almost invariably encountered after periods of cut stem culture, but in no case have losses exceeding 10 to 15 per cent. been observed. Since it seems reasonable to assume that a similar loss occurred in the leaves of all the experimental groups regardless of the solutes supplied, the values for percentage increase or decrease in nicotine content have been calculated on the basis of the number of milligrams of nicotine in the leaves of the plants supplied with tap water. In all cases in which significant increases in nicotine content have been observed in this study, the total quantity of the alkaloid in the leaves has exceeded that present in similar samples of leaves collected and analyzed at the inception of the experiments, and in most cases has exceeded the amount present in the leaf samples collected at the ends of the experimental periods. Therefore, the results of the present investigation seem to confirm the findings of KLEIN and LINSEY with respect to the ability of proline to increase the nicotine content of the leaves of excised tobacco plants.

#### THE INFLUENCE OF NICOTINIC ACID UPON THE NICOTINE CONTENT OF TOBACCO LEAVES

Assuming that excised tobacco leaves are able to synthesize nicotine directly from proline, it would seem that the supply of proline should be a limiting factor in the synthesis of the alkaloid. Furthermore, it would appear that the pyridyl portion of the nicotine molecule must necessarily be provided by the conversion of a portion of the proline itself into nicotinic acid, as suggested by TRIER and by KLEIN and LINSEY, or by preformed nicotinic acid as a naturally occurring constituent of tobacco leaf tissues. In the event TRIER's hypothesis has a basis in fact, therefore, the substitution of nicotinic acid for proline in the culture medium would be expected to have no influence upon the nicotine content of the leaves, unless, of course, the assumed reaction  $\text{proline} \rightarrow \text{nicotinic acid}$  were reversible. Since KLEIN and LINSEY do not report the results of investigations on this phase of the problem, experiments were designed to check these assumptions.

Four Connecticut no. 38 tobacco plants were placed with their cut stems



in 0.1 per cent. solutions of nicotinic acid hydrochloride. Four similar plants were placed in tap water and the entire group of cultures placed in sinks containing running water at 16° C. On the third day after the inception of the experiment, the lower leaves of the plants in water had begun to wilt. The plants in solutions of nicotinic acid hydrochloride, however, were extremely turgid and were absorbing solution at a rapid rate. At 100 hours the experiment was ended because of the complete wilting of the plants in water culture. No change was detected in the condition of the plants in nicotinic acid solutions. The leaves of the latter plants still retained the remarkable turgidity and freshness that had characterized them from the beginning of the experiment (2). In this respect, the effects of nicotinic acid upon physiological processes of tobacco leaves seemed even more pronounced than the somewhat similar effects of glutamic acid which were described earlier.

A comparison of the relative nicotine contents (table III) of the leaves receiving nicotinic acid shows an increase of nearly 16 per cent. at the end of the experimental period presumably due to the presence of the acid. The absolute quantity of volatile alkaloid present in the latter case, however, was 31 per cent. above that present in the leaves in water culture: the difference was apparently accounted for by a materially lessened dry weight loss during the culture period. It should be observed that the amount of alkaloid present in the leaves of these plants at the end of the experiment exceeded the amount present in the leaves of normal rooted plants at the end of the same period.

Further data on this phase of the problem were given by the following series of cut stem cultures. These experiments were conducted in direct light in the greenhouse in December. The previous experiments had been conducted in diffusely lighted laboratory rooms. Twelve Havana Seed tobacco plants were cut from their roots and placed in jars containing 0.013 M nicotinic acid hydrochloride plus 0.008 M proline. Two sets of 12 plants each were placed in 0.013 M solutions of nicotinic acid hydrochloride without the addition of proline. The solutions were kept chilled to a temperature of about 3° C. by continuous applications of cracked ice. The experiment was ended after the lapse of 48 hours.

Increases of 26, 26, and 32 per cent., respectively, were observed in the absolute quantity of nicotine present in the leaves after culture in solutions containing nicotinic acid hydrochloride (table V). The addition of proline to one of these culture solutions seemed in no way to influence the trend of nicotine synthesis. Dry weight increased in all of the plants under the influence of direct light exposure. The interesting feature of these results lies in the fact that the relative content of nicotine in terms of percentage of dry weight remained about constant: the increase in absolute nicotine

TABLE V

EFFECT OF NICOTINIC ACID HYDROCHLORIDE UPON THE NICOTINE CONTENT OF TOBACCO LEAVES  
AFTER CUT STEM CULTURE FOR 48 HOURS.  
LEAVES HARVESTED DECEMBER 9, 1937

EXPERIMENTAL GROUP	DRY WEIGHT OF LEAVES PER PLANT	NICOTINE PERCENTAGE	TOTAL NICOTINE PER PLANT	INCREASE IN NICOTINE	VOLUME OF SOLUTION ABSORBED PER PLANT
	<i>gm.</i>	<i>%</i>	<i>mg.</i>	<i>%</i>	<i>ml.</i>
Initial controls .....	2.8	1.62	45	- 4	
Water (no. 1) .....	3.1	1.51	47	0	93
Water (no. 2) .....	2.9	1.42	41	- 13	103
Nicotinic acid HCl plus proline .....	4.1	1.45	59	+ 26	161
Nicotinic acid HCl (no. 1) .....	4.3	1.45	62	+ 32	140
Nicotinic acid HCl (no. 2) .....	4.1	1.45	59	+ 26	147
Final controls (rooted plants)...	3.5	1.60	56	+ 19	

content in every case was directly associated with growth and increase in dry weight. Since the present experiment was conducted for only half the time usually allotted this type of experiment, it is impossible to know whether or not the action of nicotinic acid would have eventually led to an increase in the relative amount of nicotine throughout the tissues of the entire plant. It is, of course, obvious that such a condition must prevail before it can be assumed that nicotinic acid itself is capable of entering into a direct synthesis of nicotine.

From the evidence at hand, it appears that nicotinic acid possesses the ability to increase the quantity of nicotine in the leaves of excised tobacco plants. Preliminary observations, however, indicate that this relationship is not of the same type as that represented by proline and by pyrrolidone-carboxylic acid and possibly by glutamic acid. Nicotine synthesis in the presence of nicotinic acid seems to be closely related to growth and dry weight accumulation. Therefore, the increases in nicotine content may not represent a passive synthesis from nicotinic acid as a precursor but an indirect synthesis in which nicotinic acid enters into one or more important metabolic reactions before it is converted into nicotine. The similarity of this induced nicotine synthesis to the normal process of alkaloid formation in the tobacco leaf suggests important possibilities for approaching the problem of the physiological significance of nicotine in the nitrogen metabolism of tobacco.

### Summary

1. The influence of various naturally occurring organic compounds on the nicotine content of green tobacco leaves was studied by culturing excised

tobacco shoots in aqueous solutions of these substances. This was followed by quantitative analyses of the leaf tissues for changes in nicotine content.

2. The substances tested for their influences on nicotine formation were *l*-proline, *d*-glutamic acid, *l*-pyrrolidonecarboxylic acid, sodium magnesium chlorophyllin, glycine, *d l*- $\alpha$  amino-*n*-valeric acid, *d*-arginine monohydrochloride, nicotinic acid hydrochloride, *d*-glucose, and citric acid.

3. Proline, nicotinic acid hydrochloride, pyrrolidonecarboxylic acid, and glutamic acid gave indications in varying degree of possessing an effect upon the nicotine content of tobacco leaves. These results appear to confirm the observations of KLEIN and LINSER (7) and partially to support the hypothesis of TRIER (12).

4. In so far as they may be compared, the effectiveness of each of these substances in increasing nicotine content paralleled the respective closeness of its molecular structure to the nicotine molecule. That is, glutamic acid was least effective and is most remotely related to nicotine in chemical structure. Pyrrolidonecarboxylic acid was moderately effective and is somewhat more closely related to a portion of the nicotine molecule in chemical structure. Proline was most effective and is closely related structurally to one portion of the nicotine molecule. Nicotinic acid was also effective and is somewhat closely related to another portion of the nicotine molecule in chemical structure, but its effectiveness was, in these experiments at least, due almost entirely to its influence on growth and dry weight accumulation of tobacco leaves during cut-stem culture. In part, the results of this study again appear to substantiate the theory of TRIER with regard to the mechanism of nicotine synthesis *in vivo*.

5. It was shown by isolation and melting point determinations of the picrates of the volatile alkaloid complex of the various samples showing a significant increase in nicotine content that the analytical differences were probably due to increases in nicotine itself rather than to decomposition products of the substances supplied to the leaves.

6. Sodium magnesium chlorophyllin, glycine, *d*-arginine monohydrochloride, *d*-glucose, citric acid, and *d l*- $\alpha$ -amino-*n*-valeric acid gave no indications of possessing an influence upon the nicotine content of tobacco leaves.

7. Two of these substances markedly influenced the dry weight accumulation, turgidity, and growth of tobacco leaves during cut stem culture. *d*-Glutamic acid appeared to possess a sparing action on dry weight loss of tobacco leaves during cut stem culture in diffuse light and to greatly increase the water uptake and turgidity of these plants. This effect was independent of the influences of glutamic acid on nicotine content. Nicotinic acid possessed an even more marked stimulus for growth, dry weight accumulation, and increased turgidity, but this influence was associated in every case with an increase in the nicotine content of the leaves.

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# GROWTH OF WHEAT PLANTS FROM DRY AND SOAKED IRRADIATED GRAINS

EDNA LOUISE JOHNSON

(WITH FIVE FIGURES)

This comparative study of plant growth from dry and soaked irradiated seeds is in agreement with earlier experiments of the writer and of others (1) with regard to the greater sensitivity of soaked seeds to injury by x-radiation. In the experiments to be described, however, it was found that increased height over that of the controls was secured when the dry seeds were given certain doses of medium intensity. Conclusions, therefore, were not published until three series of experiments were concluded. The first and third of these were begun during successive Januaries and the second one in May.

## Methods

In the first experiment, seeds of lot I comprising 5 groups each of 100 dry Marquis wheat grains were irradiated with doses of 1000, 5000, 10,000, 20,000, 40,000, and 60,000 r-units, respectively. During treatment, all seeds were in Petri dishes, covered with cellophane. The seeds were then soaked for 18 hours, left in germinators for 12 hours, and later planted in plots in the University greenhouse. The 5 groups of lot II were similarly treated except that the period of soaking and sprouting in the germinator preceded irradiation.

Observations were made daily of the number of seedlings which showed above ground. By the sixth day after irradiation, seedlings were up in all groups of both lots, those receiving the heaviest doses appearing in diminished numbers. Beginning the tenth day after treatments and continuing weekly for four consecutive weeks and thereafter biweekly until maturity, records were taken of the height of all plants.

## Results

### SURVIVAL OF PLANTS FROM DRY AND SOAKED IRRADIATED SEEDS

A comparison of the number of seedlings living for a period of five or six weeks after irradiation indicates that doses of 1000, 5000 and 10,000 r-units given to the dry grains in no way affected their survival; 20,000 r-units reduced the number more than half, while heavier doses caused death of all seedlings within a period of 2.5 weeks. Seeds soaked before irradiation were much more sensitive to injury than the dry ones; this is shown by the fact that 5000 r-units reduced survival to approximately 5 per cent. of the total, and heavier doses caused death within 3 weeks. In the second and third experiments the procedure followed was the same as for the first, except that those doses which had proved lethal to all seedlings in a group were omitted.

Percentage of seedling survival in all three experiments for a period of 6 weeks is shown in table I and by the curves of figure 1. Data used in the

TABLE I

PERCENTAGE OF SURVIVAL FOR FIRST SIX WEEKS OF PLANTS FROM IRRADIATED SEEDS

DOSAGE	SEEDS IRRADIATED DRY WEEKS AFTER IRRADIATION			SEEDS IRRADIATED AFTER SOAKING WEEKS AFTER IRRADIATION		
	2	4	6	2	4	6
	%	%	%	%	%	%
Control						
I .....	86.0	86.0	85.0	87.0	91.0†	90.0
II .....	88.0	81.0	84.0†	87.0	82.0	79.0
III .....	95.0	96.0	96.0	100.0	100.0	100.0
Av. for 3 exp. ....	89.7	87.7	88.3	91.3	91.0	89.7
1,000 r-units						
I .....	88.0	95.0†	95.0	88.0	91.0†	91.0
II .....	83.0	80.0	80.0	84.0	81.0	77.0
III .....	97.0	95.0	96.0†	94.0	96.0	95.0
Av. for 3 exp. ....	89.3	90.0	90.3	88.7	89.3	87.7
5,000 r-units						
I .....	83.0	91.0†	95.0†	72.0	23.0	8.0
II .....	68.0	66.0	63.0			
III .....	97.0	97.0	97.0	55.0	27.0	5.0
Av. for 3 exp. ....	82.7	84.7	85.0	63.5*	25.0*	6.5*
10,000 r-units						
I .....	87.0	93.0†	89.0	39.0		
II .....	89.0	88.0	85.0			
III .....	96.0	95.0	95.0			
Av. for 3 exp. ....	90.7	92.0	89.7			
20,000 r-units						
I .....	71.0	39.0	24.0	47.0		
II .....	70.0	68.0	66.0			
III .....	88.0	76.0	70.0			
Av. for 3 exp. ....	76.3	61.0	53.3			
40,000 r-units						
I .....	53.0			25.0		
60,000 r-units						
I .....	41.0			15.0		

\* Average for first and third experiments.

† Increased number apparently due to delayed germination and growth.

chart construction are the average percentages obtained in all three experiments. In plants from the groups irradiated dry with doses up to 10,000 r-units there is little difference between experimental plants and controls. Percentage of survival was considerably reduced in plants from seeds receiving 20,000 r-units, while those with heavier doses lived not more than 2.5 weeks. During the six-weeks' period of observation injurious effects of the rays on soaked seeds were more apparent than on dry ones given the same

dose; although for groups of soaked seeds receiving as low a dose as 1000 r-units, survival was only slightly less than for the controls. The percentage of plants living from the 5000 r-units groups of soaked seeds was very low indeed, and not any of those receiving the still heavier doses lived longer than 2.5 weeks. Even during that period, they made practically no growth after pushing through the soil.

## COMPARATIVE GROWTH AT END OF SIX WEEKS

At the end of six weeks, plants from treated seeds exceeded the controls in at least two of the experiments in the following respects: (a) percentage surviving in the dry groups of 5000 and 10,000 r-units; (b) average height in the dry 1000 r-units group.

In the first two experiments, after the above data were taken, alternate plants were pulled in the following groups: controls, dry groups given the three lighter doses (1000, 5000, and 10,000 r-units), and the 1000 r-units

TABLE II

AVERAGE HEIGHT OF SEEDLINGS UP TO HEADING

DOSAGE	SEEDS IRRADIATED DRY WEEKS AFTER IRRADIATION							SEEDS IRRADIATED AFTER SOAKING WEEKS AFTER IRRADIATION						
	2	4	6	8	10	12	14	2	4	6	8	10	12	14
Control														
I .....	8.4	26.4	34.7	35.7	41.8	51.9	62.3	12.3	25.9	35.3	36.4	40.9	50.9	61.0
II* .....	14.7	36.0	55.5	71.3	81.0	.....†	.....	16.7	32.1	51.2	66.9	70.8	.....†	.....
III .....	7.9	22.6	36.1	41.1	49.5	57.4	70.4	9.4	22.4	34.9	42.1	48.7	55.5	66.5
1,000 r-units														
I .....	8.9	26.6	38.0	40.1	44.4	56.4	71.6	14.8	19.9	34.3	36.2	42.0	51.6	64.6
II .....	13.5	32.6	55.9	73.7	81.4	.....	.....	7.7	17.2	31.5	49.2	56.4	.....	.....
III .....	7.4	22.6	38.3	42.2	49.9	58.4	71.8	8.2	20.9	32.1	38.0	44.6	54.1	64.6
5,000 r-units														
I .....	8.9	27.0	37.7	41.8	46.5	55.3	64.9	1.8	4.6	11.5	13.8	22.9	42.4	47.3
II .....	13.3	34.0	54.7	75.4	84.8	.....	.....	4.1	18.5	23.1	53.0	69.0	.....	.....
III .....	6.9	21.6	36.9	42.2	52.1	60.4	70.5	1.1	3.5	23.1	25.6	30.9	43.5	57.4
10,000 r-units														
I .....	5.8	22.3	32.3	36.3	40.1	49.4	55.1	0.6	Died					
II .....	14.1	32.0	50.4	75.1	89.1	.....	.....	.....	.....	.....	.....	.....	.....	.....
III .....	5.8	21.2	35.2	41.2	49.2	56.4	66.2	.....	.....	.....	.....	.....	.....	.....
20,000 r-units														
I .....	1.3	6.1	14.9	18.8	28.9	42.0	46.1	0.5	Died					
II .....	9.9	25.8	37.5	57.2	63.4	.....	.....	.....	.....	.....	.....	.....	.....	.....
	2.3	10.4	20.8	30.0	39.2	48.9	60.2	.....	.....	.....	.....	.....	.....	.....
40,000 r-units														
I .....	0.5	Died						0.4	Died					
60,000 r-units														
I .....	0.4	Died						0.5	Died					

\* Experiment II begun in May, hence growth was more rapid than that in I and III which were begun in January.

† Experiment concluded.



group of soaked grains. The number of tillers produced in the 1000 and 5000 r-units groups of the dry grains and the 1000 r-units group of the soaked grains exceeded those of the controls. The green weight of the pulled plants in the 5000 r-units dry group was also greater than that in the controls.

#### COMPARATIVE GROWTH UP TO HEADING STAGE

The comparative effects of different doses given the dry grains will first be considered, then a similar comparison for the soaked ones will be made. Table II gives for all three experiments the biweekly measurements which

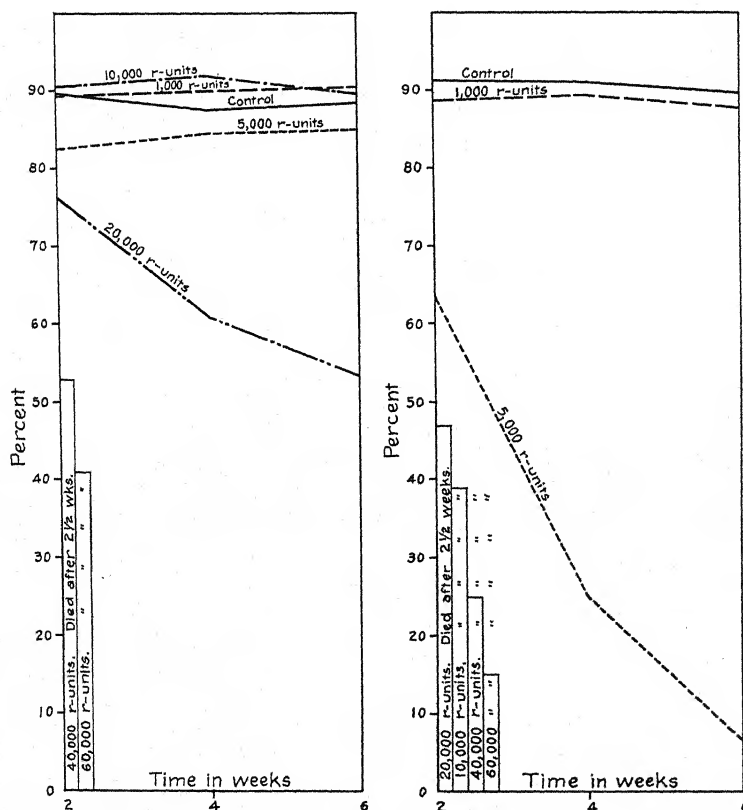


FIG. 1a. Left, survival for first 6 weeks of plants from dry irradiated seeds. Results for control and 4 lighter doses are averages from 3 experiments; others from first experiment only.

1b. Right, survival for first 6 weeks of plants from soaked irradiated seeds. Results for control and 2 lighter doses are averages from 3 experiments; others are from first experiment only.

were taken until the plants began to head out; measurements were then discontinued until maturity, when complete records were made. Plants of the

second experiment, which was begun in May, grew much more rapidly and matured two weeks earlier than those of the first and third experiments which were treated in January. Graphs were made to show heights attained at biweekly periods for all groups in the three experiments. The most representative curves were those plotted from the third experiment; they are here reproduced (fig. 2). The average height from the dry groups receiving 1000

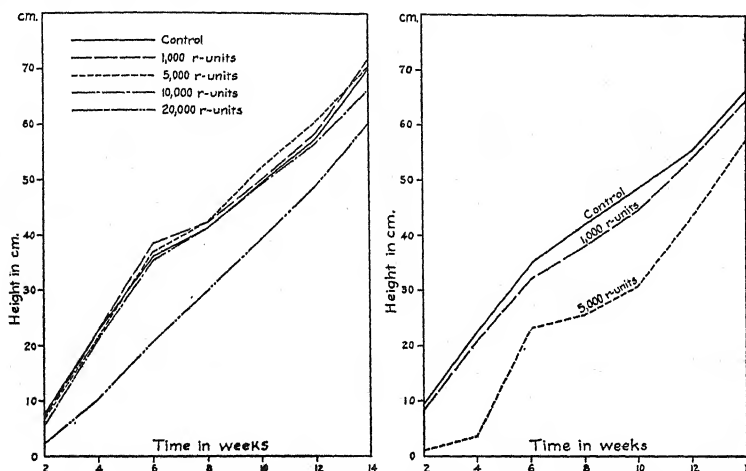


FIG. 2a. Left, height of seedlings from dry irradiated seeds until beginning of heading. Data from experiment III.

2b. Right, height of seedlings from soaked irradiated seeds until beginning of heading. Data from experiment III.

and 5000 r-units in all three experiments exceeded that of the controls; the height of plants from the 20,000 r-units dry group was considerably less. In the soaked groups, the controls averaged higher in all cases except in one experiment only; in this the 1000 r-units group measured slightly more. A dose of 5000 r-units given to soaked seeds caused considerably lessened height in the seedlings.

A comparison of the effects of the same dose upon dry and soaked seeds as judged by total height is presented graphically in figure 3. This was constructed from the height of plants of series I measured at biweekly periods; the results, however, were much the same in the other two experiments as well. To simplify the graph, only three groups were used in its construction. The vigorous growth made by plants from seeds irradiated dry with 5000 r-units exceeded the growth of the controls. This was true not only for plants of experiment I, which is used in the graph, but for all three experiments. Growth from the soaked grains, given the same dose was very much less. As judged by height of seedlings, the amount of injury to the soaked 5000 r-units group was approximately the same as that to the 20,000 r-units dry

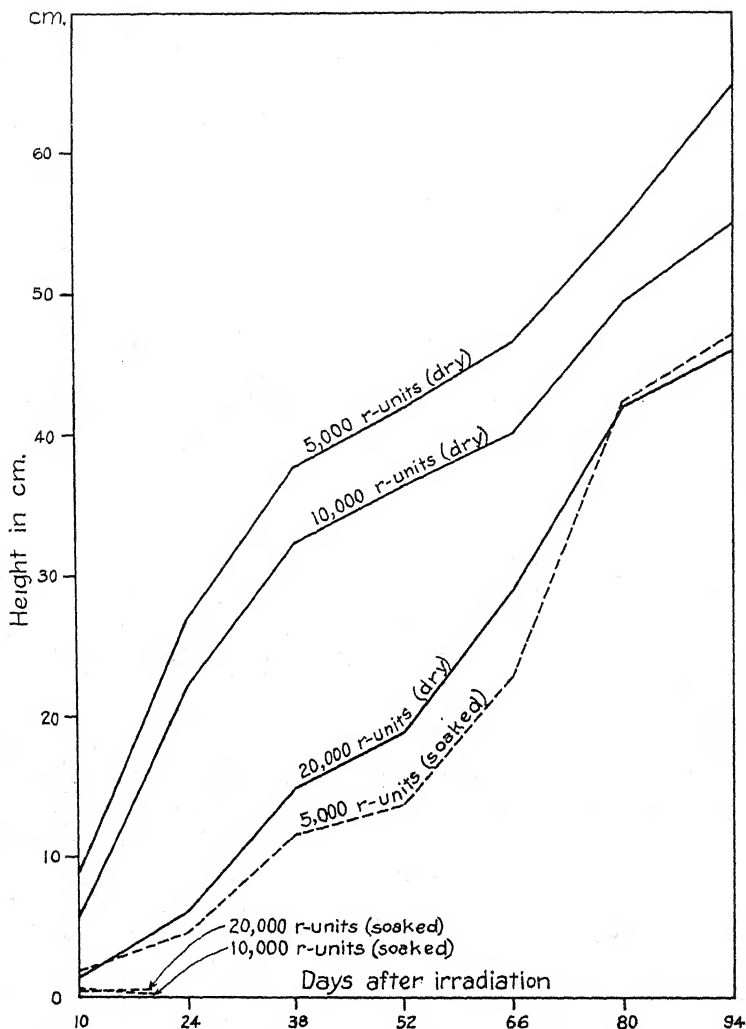


FIG. 3. Comparison of plant growth from irradiated dry and soaked seeds. Data from experiment I.

group. Plants from dry grains given 10,000 and 20,000 r-units show less growth, while the soaked seeds receiving these doses made practically no growth, and were dead by the 20th day after treatment.

#### GROWTH AT MATURITY

At the conclusion of the experiment, records were taken of (a) the height including head; (b) the height from stem base to the tip of the highest leaf. In addition to these measurements tillers were counted, and the percentage of

tillers producing heads was determined. The green and dry weights were also recorded. From table III which gives data for all three experiments,

TABLE III  
GROWTH OF PLANTS AT MATURITY

DOSAGE	No. OF PLANTS	AV. HEIGHT INCLUDING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	TILLERS PRODUCING HEADS	AV. FRESH WEIGHT	AV. AIR-DRY WEIGHT*
<i>Seeds irradiated dry</i>		cm.	cm.		%	gm.	gm.
Control							
I .....	42	102.1	92.2	3.2	68.4	16.8	4.8
II .....	42	81.0	72.1	2.0	72.3	6.9	2.3
III .....	95	91.9	85.5	2.6	76.4	9.5	.....
1,000 r-units							
I .....	46	107.8	98.8	3.4	75.3	20.0	7.0
II .....	42	87.4	73.7	1.8	77.3	6.0	2.2
III .....	96	94.2	88.3	2.7	62.5	9.0	.....
5,000 r-units							
I .....	47	107.5	93.8	5.2	76.5	32.2	10.3
II .....	32	87.4	75.6	2.5	71.2	8.2	2.8
III .....	96	98.1	86.7	2.8	85.0	8.4	.....
10,000 r-units							
I .....	49	93.3	80.6	4.6	75.9	23.7	7.6
II .....	44	89.1	76.2	2.5	81.6	8.2	2.6
III .....	95	94.4	84.2	2.5	67.2	8.4	.....
20,000 r-units							
I .....	19	112.6	92.8	5.8	81.1	31.0	10.6
II .....	33	63.4	62.1	2.0	81.5	4.4	1.5
III .....	56	84.6	79.6	3.5	69.3	1.1	.....
<i>Seeds irradiated after soaking</i>							
Control							
I .....	47	106.6	92.0	2.5	75.4	14.1	4.5
II .....	42	70.8	65.9	1.8	78.9	5.2	1.8
III .....	100	91.7	82.0	3.0	63.2	9.4	.....
1,000 r-units							
I .....	44	99.3	91.4	3.0	65.6	14.3	4.6
II .....	35	56.4	60.8	3.2	44.6	5.9	1.4
III .....	91	98.8	86.7	2.6	69.3	9.5	.....
5,000 r-units							
I .....	3	91.7	79.0	2.7	75.0	13.3	4.2
II .....	1	47.0	69.0	9.0	11.0	10.5	3.1
III .....	4	93.6	81.4	9.2	78.4	47.9	.....

\* Owing to an accident, accurate weights were not obtained for experiment III.

the percentage increase over the control was calculated for the first three items listed above for the dry groups. These are presented graphically in figure 4.

Dry-seeded groups receiving 1000 or 5000 r-units in all three experiments exceeded the controls in average height of plants including head, as well as in

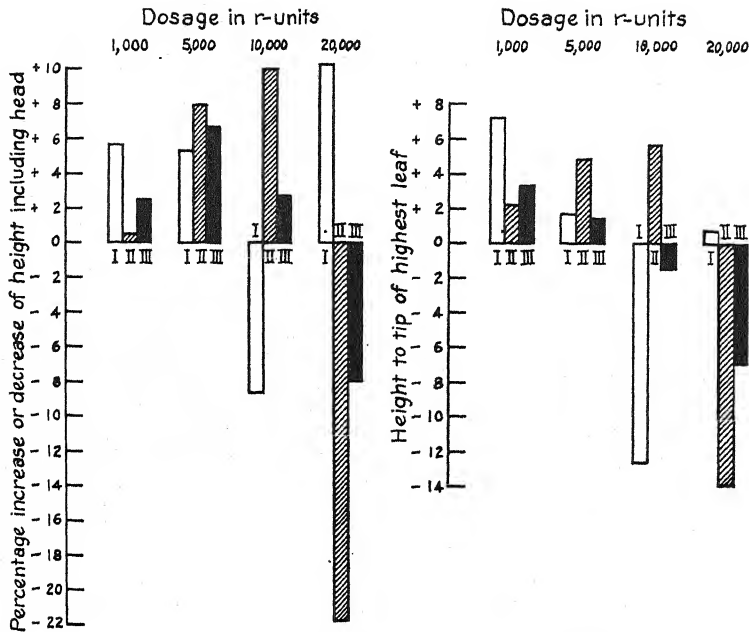


Fig. 4. Percentage increase or decrease over the controls of plants from irradiated dry seeds.

average height to the tip of the highest leaf. In the 10,000 r-units group, in two out of three experiments, the height exceeded that of the controls; this occurred in but one experiment with the 20,000 r-units group.

Since increase in branching is one of the usual results obtained by x-raying plants, it is not surprising to find more tillering in every group surviving the given dose. Figure 5 gives the percentage increase in tillering over the controls. A very considerable increase in the dry groups given 5000 r-units and 10,000 r-units is shown. As pointed out earlier in this paper, the number of plants in the 20,000 r-units group was much reduced, hence the increased space in the plot available because of fewer plants was perhaps a factor in causing increased tillering. Few of the soaked group exceeded the controls in any respect at maturity except in tillering. In the first two experiments, groups receiving 1000 and 5000 r-units respectively showed a great increase in tillering over controls (table III).

Table V shows all the various respects in which at maturity the performance of plants from the irradiated grains exceeded that made by the controls. Roman numerals are used to indicate the experiment in which the growth of experimental plants was greater than that of the controls. There is complete agreement in the results of the three experiments regarding the increase over the control in height and tillering in the 1000 and 5000 r-units

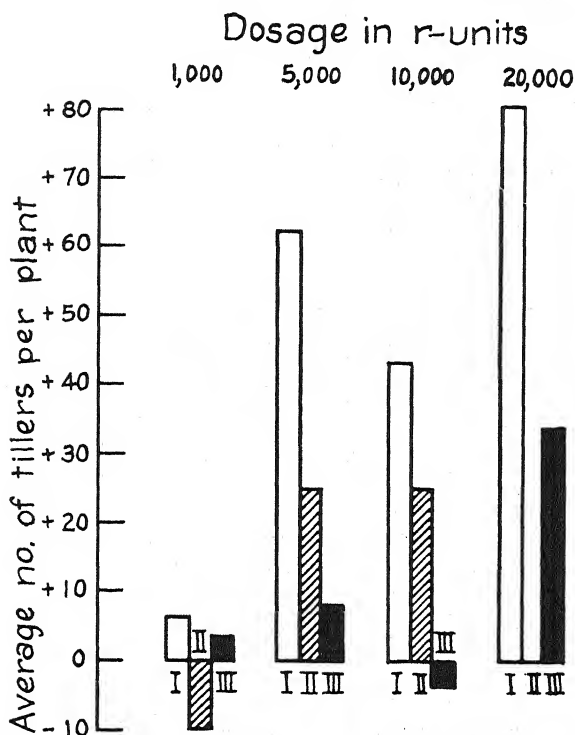


FIG. 5. Percentage increase or decrease over the control of tillering in plants from irradiated dry seeds.

group. This increase, however, does not result in increased green or dry weights in every case, notably in the third experiment. Hence although doses of 1000, and particularly 5000 r-units, apparently increase height and tillering, disagreement as to increase in green and dry weight negatives any claim that these doses cause a stimulation which results in actual increase of plant materials.

### Discussion

The procedure followed in these experiments with regard to length of the soaking period for the seeds and the heaviest dose employed, is similar to that used by SUGIURA (3) in a study in which he determined the effects of x-rays on the vitamin B content of wheat seedlings. SUGIURA found that the height of 20-day seedlings from seeds irradiated wet was twice as great as from those irradiated dry, hence he concludes that "wheat seeds in dry, dormant state are more sensitive to x-rays than those in wet physiological state as regards growth of plants after irradiation when doses over 60,000 r-units are given."

TABLE IV

PERCENTAGE INCREASE OR DECREASE OF PLANTS FROM IRRADIATED SEEDS OVER CONTROL

DOSAGE	AV. HEIGHT INCLUD- ING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	TILLERS PRODUC- ING HEADS	AV. FRESH WEIGHT	AV. AIR- DRY WEIGHT*
<i>Seeds irradiated dry</i>	%	%	%	%	%	%
1,000 r-units						
I .....	+5.6	+7.2	+6.2	+10.1	+19.0	+45.8
II .....	+0.5	+2.2	-10.0	+6.9	-13.0	-4.3
III .....	+2.5	+3.3	+3.8	-18.2	-5.3	.....
5,000 r-units						
I .....	+5.3	+1.7	+62.5	+11.8	+91.7	+114.6
II .....	+7.9	+4.8	+25.0	-1.5	+8.8	+21.7
III .....	+6.7	+1.4	+7.9	+14.8	+11.6	.....
10,000 r-units						
I .....	-8.6	-12.6	+43.7	+11.0	+41.1	+62.5
II .....	+10.0	+5.7	+25.0	+12.9	+18.8	+13.0
III .....	+2.7	-1.5	-3.8	-12.0	-11.6	.....
20,000 r-units						
I .....	+10.3	+0.7	+81.2	+18.6	+84.5	+120.8
II .....	-21.7	-13.9	0.0	+12.7	-36.2	-34.8
III .....	-7.9	-6.9	+34.6	-9.3	-88.4	.....
<i>Seeds irradiated after soaking</i>						
1,000 r-units						
I .....	-6.8	-0.7	+20.0	-13.0	+1.4	+2.2
II .....	-20.3	-7.0	-77.8	-43.5	+13.5	-22.2
III .....	+7.7	+5.7	-13.3	+9.7	+1.1	.....
5,000 r-units						
I .....	-14.0	-14.1	+8.0	-0.5	-5.7	-6.7
II .....	-33.6	+4.7	+400.0	-85.9	+101.9	+72.2
III .....	+2.1	-0.7	+206.7	+24.1	+388.3	.....

\* Owing to an accident, accurate dry weights were not obtained for experiment III.

Experimenters are agreed that very heavy irradiation of seeds does not prevent germination and some growth. However, practically all have found that heavier doses are required to inhibit growth of dry seeds than soaked ones. According to the experiments described in this paper, doses of 40,000 r-units for the dry seeds and 10,000 r-units for the soaked ones cause cessation of growth after sprouts less than 1 cm. in height have appeared. The writer, therefore, agrees with HENSHAW and FRANCIS (2) that growing material is more strongly affected than non-growing by equal doses of radiation given in the same way.

### Summary

Results upon growth of wheat plants are presented from three sets of experiments with dry and soaked seeds exposed to x-ray doses ranging from

TABLE V

PLANTS FROM IRRADIATED GROUPS WHICH EXCEEDED CONTROLS AT MATURITY  
(ROMAN NUMERALS REFER TO THE NUMBER OF THE EXPERIMENT)

DOSAGE	AV. HEIGHT INCLUD- ING HEAD	AV. HEIGHT TO TIP OF TALLEST LEAF	AV. NO. OF TILLERS PER PLANT	PER- CENTAGE OF TIL- LERS PRO- DUCING HEADS	AV. FRESH WEIGHT	AV. AIR-DRY WEIGHT
<i>Seeds irradiated dry</i>						
1,000 r-units .....	I II III	I II III	I III	I II	I	I
5,000 r-units .....	I II III	I II III	I II III	I	I II	I II
10,000 r-units .....	II III	II	I II	I II	I II	I II
20,000 r-units .....	I	I	I III	I II	I	I
<i>Seeds irradiated after soaking</i>						
1,000 r-units .....	III	III	I II	III	I II III	I
5,000 r-units* .....	III	II	I II III	III	II III	II

\* Survival so low that results are not comparable.

1000 to 60,000 r-units. Comparisons are made with controls kept under the same environmental conditions.

1. The percentage of seedlings surviving in the groups treated dry with 1000, 5000 and 10,000 r-units respectively was as high as in the control. A dose of 20,000 r-units, however, reduced the number to less than half; heavier doses applied to dry seeds caused the death, within 3 weeks, of all seedlings. Seeds soaked before irradiation were much more sensitive to injury than dry ones; the seedlings from grains treated with doses heavier than 5000 r-units all died within 3 weeks.

2. Tillers on 6-week-old seedlings from irradiated dry grains were more numerous in 1000 and 5000 r-units groups than in the controls. At maturity, with these same doses, the tillering was increased and also the height, including head, and the distance to the tip of the highest leaf. In the 5000 r-units group, in two of the experiments, the green weight of the plants exceeded that of the controls.

3. Dry grains treated with 10,000 r-units in two out of three experiments increased average height, tillering, percentage of tillers bearing heads, and green and dry weight.

4. Seedlings from soaked grains made less growth in all respects than did the controls, except that the 1000 r-units group tillered more than the controls in two of the experiments, and in the 5000 r-units group in all experiments tillering was considerably increased.

Acknowledgment is made to the Committee on the Effects of Radiation upon Living Organisms of the National Research Council for assistance in



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# GREENHOUSE STUDIES OF THE EFFECT OF CLIPPING THE TOPS OF ALFALFA AT VARIOUS HEIGHTS ON THE PRODUCTION OF ROOTS, RESERVE CARBO- HYDRATES, AND TOP GROWTH<sup>1</sup>

C. M. HARRISON

(WITH FOUR FIGURES)

Alfalfa is frequently used as a pasture crop in the state of Michigan, such use being particularly advantageous during the hot summer period. Grazing of livestock on alfalfa generally removes the upper portions of the top growth of the plant first, the grazing being practically uniform over the entire area. This makes it possible to remove the livestock from an alfalfa field before all of the top growth is consumed, or to stock the area so that the basal portions of the shoots are rarely eaten. Management practices which permit prolonged close grazing frequently result in considerable damage to the stands.

In an attempt to determine the amount of top growth necessary to keep alfalfa plants from being weakened, or the reserves of stored food from being depleted, cultures of Hardigan alfalfa plants were set up in the greenhouse at Michigan State College in East Lansing on October 9, 1936. The cultures were grown in quartz sand in 10-inch clay pots from seedling plants collected in the field and transplanted to pots. Eight plants were placed in each pot, and each treatment was replicated five times. All cultures were supplied with the same nutrient solution.

On March 29, 1937, the plants had reached the blooming stage and three cultures were photographed (fig. 1), harvested, and the green and dry weights of tops and roots determined. These three cultures were harvested before any cutting treatments were begun and will be known as "initial" checks. After the roots were washed free of sand a small segment from each of 15 roots was placed in a fixing solution of formalin alcohol to be preserved for histological study of stored starch. The cultures remaining were grouped into lots of 5 and cut weekly from March 31st until May 26th, when the experiment was discontinued. Five cultures were left uncut throughout the duration of the experiment and were harvested when the experiment was completed. These 5 cultures will be known as "final" checks. In the case of the final checks the alfalfa in the greenhouse had reached a condition comparable to that in the field when alfalfa is ordinarily cut for hay. The yield of green and dry weight of tops was determined for each culture within each cutting treatment. The cutting treatments were as follows:

<sup>1</sup> Contribution from the Section of Farm Crops, Michigan Agricultural Experiment Station, East Lansing, Michigan. Journal Article no. 320 (new series).

1. Cut to 1 inch weekly.
2. Cut down gradually for 4 successive weeks until a 2-inch height was attained, after which this 2-inch height was maintained.
3. Cut to 6 inches weekly.
4. No cutting, or "final" checks.

The production in grams of green and dry material from the cultures is recorded in table I.

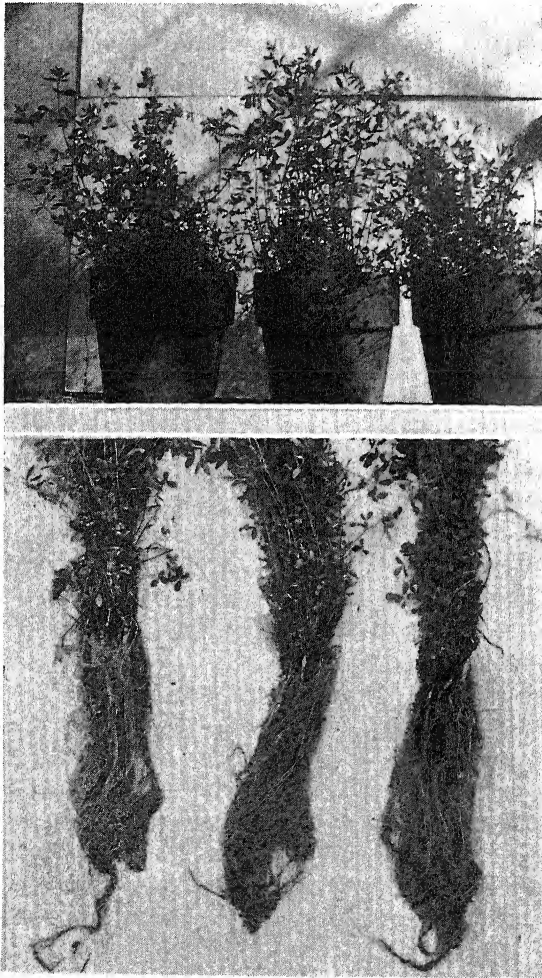


FIG. 1. Cultures of alfalfa plants before cutting was begun on 3/29/37. Above, before washing sand from roots. Below, after sand was washed from roots.

# PRODUCTION OF ALFALFA IN GREEN AND DRY WEIGHTS PER POT WHEN CUT AT DIFFERENT HEIGHTS

[illegible]

TABLE I—(Continued)

A study of the table shows that the cultures cut to one inch yielded approximately as much as did those cut to 6 inches for the two weekly periods following the initial cutting. On April 21st, however, three weeks after the initial cutting, the cultures cut at 6 inches produced more new growth above the point of clipping than did the cultures cut to one inch. This same relation held true until the close of the experiment. On May 19th, one week before the final harvest date, the cultures cut at 6 inches were producing approximately 6 times as much new growth as were the cultures cut to one inch. It is difficult to compare the cultures, cut gradually down to a 2-inch height, with those subjected to the other two cutting treatments. The cultures cut gradually to 2 inches were clipped shorter each week up to the fourth week, when they were finally cut down to two inches. Consequently the production recorded for these cultures was not only "recovery" growth, but also a certain portion of the original top. It will be noted that these cultures produced more dry weight than those cut to one inch continuously except on the 28th of April, which was the date following the last removal down to two inches. Toward the end of the experiment, these cultures were producing less "new growth" above the point of cutting than were those cut to 6 inches, an indication that the reserves were being depleted in the cultures cut at two inches.

After the initial cutting date, growth in the cultures cut to one inch was very meager. The average total production of dry matter per pot in the top growth was only slightly greater than that secured from the initial

TABLE II

AVERAGE PRODUCTION IN DRY WEIGHT PER POT FOR VARIOUS CUTTING TREATMENTS

TREATMENT	TOTAL PRODUCTION OF DRY MATTER INCLUDING FIRST AND LAST CUTTINGS	YIELD IN PERCENTAGE OF INITIAL CHECKS	INCREASE IN PRODUCTION OF DRY MATTER DURING PERIOD OF CUTTING WHEN COMPARED TO INITIAL CHECKS†	PRODUCTION COMPARED TO FINAL CHECKS UN CUT UNTIL MAY 26
	<i>gm.</i>	<i>%</i>	<i>gm.</i>	<i>%</i>
Initial checks March 29*	22.7	100	Not cut after March 29	
Final checks on last cutting date 5/26 .....	45.1	198	22.4	100
Cut to 1 inch weekly 3/31 to 5/26 .....	25.8	113	3.1	13.8
Cut to 2 inches gradually 3/31 to 5/26 .....	42.1	185	19.4	86.7
Cut to 6 inches weekly 3/31 to 5/29 .....	55.9	246	33.2	148.4

\* March 29 was the date when the various cutting treatments were started.

† This represents dry matter removed in various cutting treatments after March 29.

check pots on March 29th, when the cutting treatments were begun. The production of top growth secured from the cultures cut gradually to two inches was approximately twice that of the initial checks and almost equal to the production of the final checks cut only on May 26th. The average total production per pot of the cultures cut to 6 inches was almost three times as much as that of the initial checks, more than double that of the cultures cut to one inch, and approximately 25 per cent. greater than that of the cultures cut to two inches or of the final checks.

Table II summarizes the production of the different cutting treatments.

The percentage of dry matter in the tops was approximately equal for all the cutting treatments.

When the cultures were finally harvested, on May 26th, the sand was washed carefully from the roots, and the green and dry weights of the roots were determined and recorded in table III. Photographs were taken of an individual culture from each treatment before harvesting, and after the



FIG. 2. Alfalfa cultures cut weekly for 8 weeks, before and after washing sand from roots. Left to right, cut to one inch, to two inches gradually, to 6 inches, and uncut check.

TABLE III  
GREEN AND DRY WEIGHTS OF ALFALFA ROOTS FROM DIFFERENT CUTTING TREATMENTS

TREATMENT	Pot I		Pot II		Pot III		Pot IV		Pot V		AVE.		PRODUC- TION OF DRY MAT- TER OF ROOTS COM- PARED TO INITIAL CHECK	DRY MATTER	PERCE NAGE O F DRY MA TER IN ROOTS COMPAR ED TO INITI AL CHECK
	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT	GREEN WEIGHT	DRY WEIGHT			
Initial check before cut- ting was begun 3/29	63.8	11.7	90.8	16.0	84.7	17.5	gm.	gm.	gm.	gm.	79.8	15.1	100.0	18.9	100.0
Cut to one inch weekly	57.0	9.5	50.0	8.5	33.0	4.5	34.0	5.0	71.0	10.5	49.0	7.6	50.0	15.5	82.0
Cut to two inches gradually	141.0	23.0	107.0	21.0	68.0	14.5	84.0	12.0	126.0	25.5	105.2	19.2	127.0	18.1	95.0
Cut to 6 inches weekly	206.0	49.5	118.0	23.5	172.0	51.0	168.0	39.5	217.0	55.0	176.0	43.7	288.0	24.8	131.0
Final checks no cutting	230.0	63.0	138.0	34.0	131.0	37.5	215.0	66.5	143.0	41.5	171.0	48.5	323.0	28.3	149.0



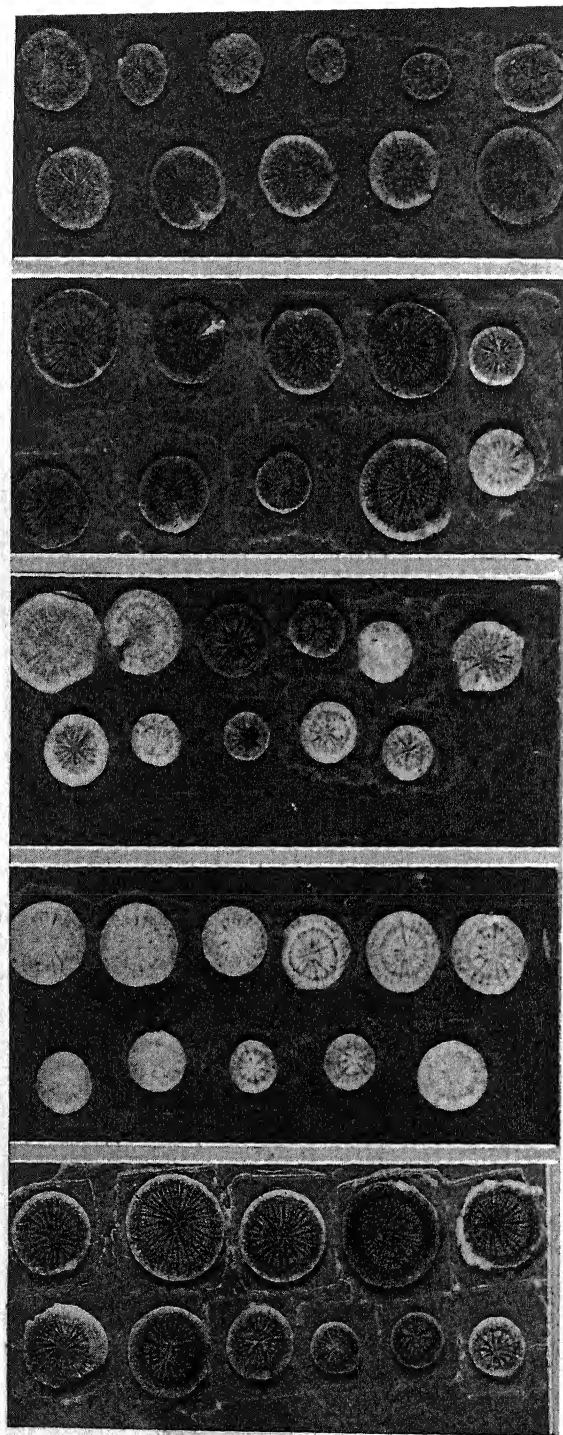


FIG. 3. Photograph of eleven individual alfalfa roots from each of the various cutting treatments showing stored starch. Top to bottom, checks at the beginning of the experiment on 3/29/37; after cutting to 6 inches for 8 consecutive weeks; after cutting to two inches gradually; after cutting to one inch; and checks at the close of the experiment on 5/26/37 ( $\times 1\frac{1}{2}$ ).

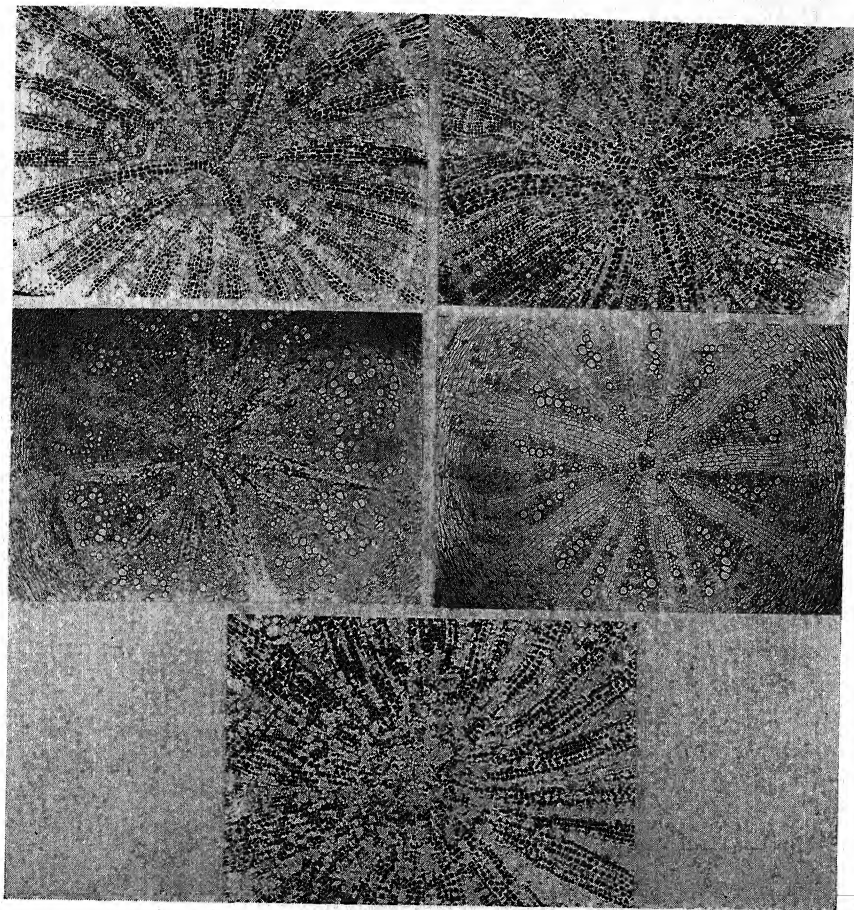


FIG. 4. Photomicrographs of alfalfa roots from the various treatments showing stored starch. Left to right: Top, check on 3/29/37, and after cutting to 6 inches for 8 consecutive weeks; center, after cutting to two inches gradually, and to one inch for the same period; bottom, check at close of experiment on 5/26/37 ( $\times 50$ ).

sand was washed from the roots, in order to note the quantity of roots present (fig. 2). A small segment of approximately 15 roots from each treatment was fixed in formalin alcohol for histological study.

It will be noted from a study of table III that the average dry weight of the roots from the cultures cut to one inch continuously was approximately half as great as the weight of roots harvested before cutting was begun. The dry weight of roots from the cultures cut gradually to two inches was only slightly greater than the weight of the roots of the original checks, while the roots of the cultures cut to 6 inches, and those of the final checks, had a dry weight practically three times as great as that of the initial

checks. It seems that cutting at one inch not only prevented the growth of new root tissue and accumulation of reserve materials, but also brought about a loss of root tissue present when cutting was begun. Roots of the cultures cut gradually to two inches increased only slightly in dry weight over that of the initial checks, while the 6-inch cutting treatment allowed the production of root growth almost as great as that of the final checks. A comparison of the average total production and the percentage dry matter in the roots of the various treatments is shown in table III. If the percentage of dry matter in the roots before cutting was begun is given the value of 100, the figures in the last column of the table give the comparative values for the other treatments. Substantial reductions may be noted in the case of the tops which were cut at one or two inches. Increases are found in the case of the plants which were uncut or cut at 6 inches.

This variation in percentage of dry matter indicated a difference in the quantity of material stored in the roots from the various treatments. In order to study this condition the roots fixed at the final harvest date, and also those fixed before cutting was begun, were imbedded by the paraffin method and sections made at 15  $\mu$ . A section from each of 11 roots from each treatment was mounted on a slide, stained with Gram's iodine solution and photographed. Photomicrographs were likewise made of one root from each treatment, selected as representative of its group. Figures 3 and 4 show the comparative differences in stored starch of the roots from the various treatments. The roots of the plants cut to one inch continuously are seen to have little if any starch remaining, while those from the culture cut gradually to two inches have had their supply reduced (about half of the roots are still quite well supplied, whereas in the other half the starch is practically gone). The roots from the original checks, the final checks, and from the plants cut at 6 inches continuously show an abundant storage of starch.

### Discussion

When the top growth of alfalfa plants was removed weekly at specified levels for a period of 8 successive weeks, various responses were noted in the production of new top and root growth and root storage, as compared with the responses of uncut cultures. Marked reductions were found in all respects in cultures cut at one inch; somewhat less prominent reductions occurred in cultures cut at two inches. Cutting at 6 inches appeared to interfere only slightly with the functioning of the plant in the production of top and root growth and stored material as compared with the uncut cultures.

In pasturing alfalfa, close removal of top growth is often allowed. This practice brings about a reduction in the production of dry matter by the plants, as well as the depletion of any previously stored reserve materials.

Unless given an opportunity to grow and replenish these reserves the plants may die. Frequently the plants die during the growing season, although the hazards to the stand are particularly severe if stored food has been depleted just previous to freezing weather. Reduction of the stand under close grazing actually occurs in field practice, whereas very little difficulty is encountered if the alfalfa is allowed to retain from 6 to 10 inches of top growth during the grazing season. It may be assumed that the close and frequent removal of the top growth of alfalfa in the greenhouse tests is an indication of the conditions brought about by too close grazing in the field and that the maintenance of a reasonable top growth is necessary to keep alfalfa in fairly good condition. Grazing to one or two inches may so deplete the materials stored in the roots that the plants will be weakened and, unless given a chance to replenish their food reserves, will die from carbohydrate starvation or be more subject to winter injury.

In many of the schemes of pasture management which have been proposed, there has been a pronounced tendency to insist that plants suitable for grazing must be so constituted physiologically that they will "stand abuse,"—in particular, close grazing. But plants which survive best under close grazing are not always most productive in a given environment. If the results reported here are reasonably indicative of field performance, it appears entirely feasible to so manage the grazing of alfalfa as to secure top growth production and storage of food reserves in the roots comparable to that obtained when the crop is well managed for hay.

In the event of the general application of this principle to other plants, it seems reasonable to venture that the development of different schemes of pasture management, suited to the physiology of existing plants of superior productive merit, may be at least as promising a means of pasture improvement as the selection of special types of plants which may withstand the "abuse" of a fixed system of management based on close grazing.

### Summary

1. Alfalfa cultures grown in the greenhouse were cut to one inch, two inches gradually, and to six inches continuously for eight successive weeks from March 31st to May 26th.
2. Subsequent growth of tops was materially lessened in cultures which were cut to one inch, in comparison to those cut gradually to two inches and those cut to six inches.
3. The percentage of dry matter of top growth from the various treatments was approximately equal.
4. Cultures of alfalfa plants which were cut to one inch weekly for eight consecutive weeks had roots at the close of the experiment which contained only 50 per cent. as much dry matter as they did when the cutting

was begun, and approximately 14 per cent. as much as was found in the roots of plants not cut until the close of the experiment. These uncut plants had developed in the greenhouse to a maturity comparable to that of alfalfa in the field when it is commonly cut for hay.

5. In cultures which were cut to two inches gradually, the average dry weight of roots remained approximately equal to that which prevailed when cutting treatments were begun, but they produced only 40 per cent. as much dry matter as the alfalfa harvested only at the close of the experiment.

6. In cultures cut to 6 inches each week for 8 consecutive weeks, the average dry weight of the roots was approximately equal to that of the cultures harvested only at the close of the experiment, was three times as much as that of the checks at the beginning of cutting treatments, and six times as much as that of the cultures cut to one inch.

7. The stored starch in the roots from cultures cut to one inch was entirely depleted, that in the cultures cut gradually to two inches had been partially depleted, whereas the roots sampled at the beginning of the cutting treatments, the roots of cultures cut to 6 inches and the roots of cultures cut only once, at the end of these trials, had an abundant supply of starch.

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# EQUIPMENT FOR THE GROWING OF PLANTS AT CONTROLLED TEMPERATURES<sup>1</sup>

E. MARION BROWN

(WITH SEVEN FIGURES)

## Introduction

Equipment designed for growing plants under conditions of controlled temperature was installed at the Missouri Experiment Station, Columbia, Missouri, in the fall of 1936. Some features of this apparatus are modifications of temperature control equipment devised and used by other workers. Other features, particularly the use made of standard air-conditioning equipment, are believed to represent new developments in apparatus for the growing of plants at controlled temperatures. The description which follows is presented with the idea that other investigators might make use of certain features in the designing of other equipment for the growth of plants in controlled environments.

## Description of apparatus

The temperature control equipment described herein consists of 3 growth chambers, the devices necessary for the regulation of temperature in them, and the greenhouse in which they are housed. The greenhouse, 15 feet wide by 28 feet long, is oriented so that its long axis runs east and west. The growth chambers are located along its south side, with a space of approximately 6 feet left between the end compartments and the end walls of the greenhouse, an arrangement which provides equal lighting for all compartments. All three growth chambers operate simultaneously with air and soil temperature independently controlled.

A general view of the greenhouse and the temperature control equipment which it contains is shown in figures 1 and 2.

## GROWTH CHAMBERS

Each growth chamber consists of a soil temperature tank and an air chamber. The soil temperature tank occupies all of the compartment, except a 6-inch space between the tank and rear wall, to a height of 3 feet above the floor. The air chamber is that part of the compartment above the soil temperature tank. This space is limited at the top and on the south side by a portion of the roof and south wall of the greenhouse.

The front and side walls of each air chamber, and the doors in the rear,

<sup>1</sup> Contribution of the Division of Forage Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Missouri Agricultural Experiment Station, Columbia, Missouri.



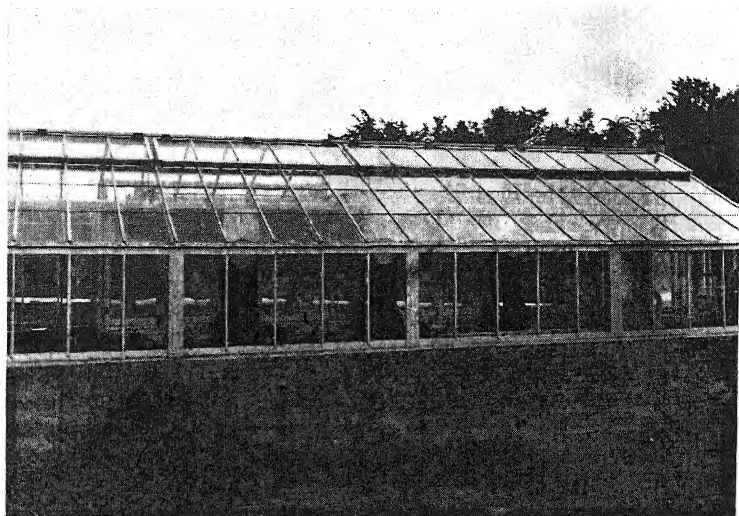


FIG. 1. Greenhouse containing the temperature control units as viewed from north.

are fitted with double panes of glass, separated by a 1-inch air space to reduce heat loss. The roof, however, is covered by a single thickness of standard greenhouse glass, in order to admit light with a minimum of absorption by the glass. The partitions and rear walls of the compartments below the air chamber consist of double walls of masonite, the panels of which are separated

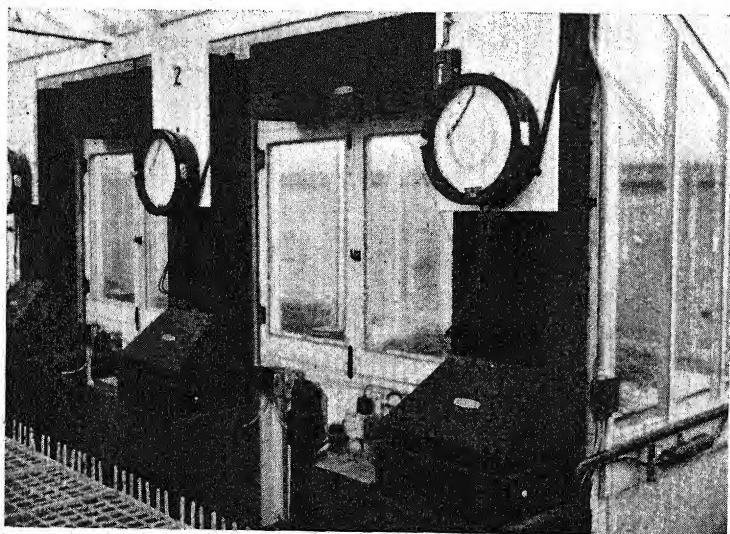


FIG. 2. Interior of greenhouse showing temperature control units.

by a 2-inch air space. The concrete side wall of the greenhouse forms the front wall of each compartment below the air chamber.

The foregoing description, as well as that which follows, will be clarified by reference to the drawings presented (figs. 3 to 6). Dimensions of the greenhouse and growth chambers are shown in figures 5 and 6.

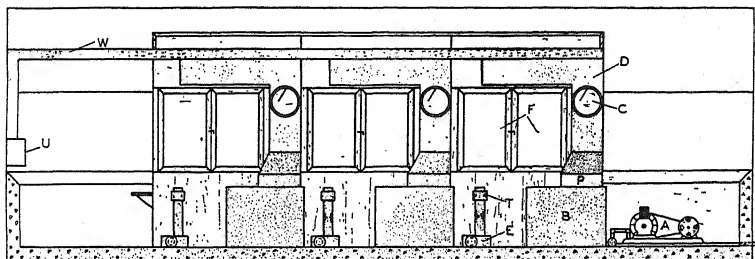


FIG. 3. Longitudinal section of greenhouse showing location and arrangement of temperature control units. A, compressor; B, air cooling unit; C, recording temperature controller; D, air duct; E, rotary pump and motor; F, doors to air chamber; P, air heater; T, immersion heater; U, relays operating heaters; W, electrical conduit.

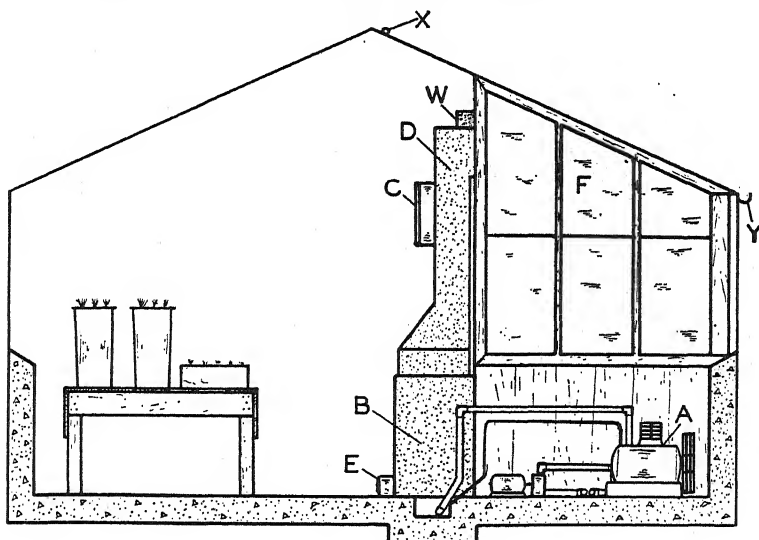


FIG. 4. Cross-section of greenhouse containing temperature control units. A, compressor; B, air cooler; C, recording temperature controller; D, air duct; E, motor operating rotary pump; F, glazed wall of air chamber; W, electrical conduit; X, roof irrigator; Y, gutter.

#### MAINTAINING CONSTANT AIR TEMPERATURES

The air temperature is held constant by continuously circulating the air through the air chamber and the temperature regulator just outside and back



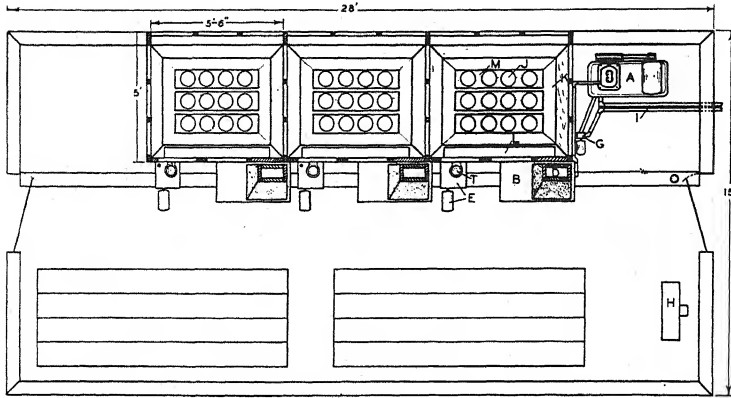


FIG. 5. Floor plan of greenhouse and temperature control units. A, compressor; B, air cooler; D, air duct; E, G, rotary pumps and motors; H, unit heater (steam); I, water pipes to cooling tower; J, soil container; K, soil temperature tank; L, baffle over air outlet; M, sleeve cover.

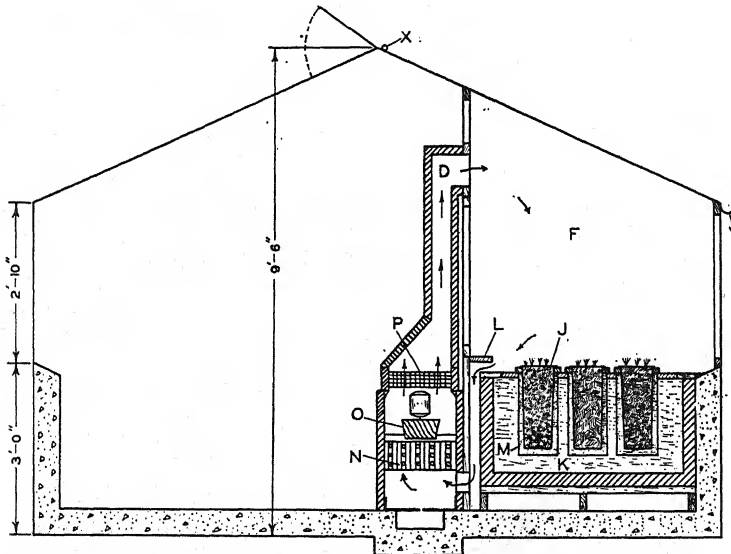


FIG. 6. Cross-section of greenhouse and temperature control unit. D, air duct; F, air chamber; J, soil container; K, soil temperature tank; L, baffle over air outlet; M, sleeve; N, cooling coils; O, fan; P, electric heater.

of each compartment (B and P, figs. 3 to 5). The course followed by the circulating air, as well as the essential parts of the temperature regulator, are shown in the cross-section of greenhouse and temperature control unit presented in figure 6. The air, withdrawn from the air chamber (F, fig. 6)

under the baffle (L, fig. 6), passes between the soil temperature tank and rear wall of the compartment into the lower part of the temperature regulator, through a short air duct. As the air passes through the temperature regulator it is either heated or cooled, depending on its temperature as it leaves the air chamber, relative to that which the controls are set to maintain. The heated or cooled air is returned to the air chamber through the air duct (D, fig. 6), the outlet of which extends nearly the full width of the air chamber near the top. This outlet is provided with curved deflectors placed to give a uniform distribution of the inflowing air throughout the air chamber.

The cooling unit of the air temperature regulator is a Frigidaire room cooler, Model C-100-B. It consists of a cabinet containing refrigeration coils provided with fins to increase their cooling surface (N, fig. 6) and a fan which circulates the air (O, fig. 6). The heating unit is a Chromalox Air Blast Heater, Model CAB-5, mounted in the air duct just above the cooling unit. Each heater is divided into two equal 1500-watt, 220-volt, 3-phase circuits, either of which can be switched on independently of the other, to increase or decrease the amount of heat supplied to the air. The heaters are equipped with thermo cut-outs to break the circuit in case of overheating. The entire air temperature regulating unit and air duct leading from it to the air chamber are covered by a one-inch layer of cork for insulation.

In order to reduce the absorption of heat by the air chamber and lighten the cooling load during warm, clear days, a sheet of water is run over the south slope of the greenhouse roof. A  $\frac{3}{4}$ -inch copper pipe with small nozzles inserted at 6-inch intervals extends the full length of the roof just below the ridge (X, figs. 4 and 6). By this means three streams of water, each approximately  $\frac{1}{20}$  inch in diameter, are directed against each 16-inch pane of glass at an angle which spreads the water in a thin sheet over the full width of the sloping glass roof. The water is drained away by a gutter along the south eaves (Y, figs. 4 and 6). Tap water, having a summer temperature of 60° to 70° Fahrenheit is used for this purpose and is not recirculated.

#### SOIL TEMPERATURE TANK

The soil temperature tank (K, fig. 6) consists of an iron tank, insulated by a 2-inch layer of cork which is in turn covered by an outer sheath of lighter material. Built into each tank are three iron sleeves (M, fig. 6), each 10 inches wide, 40 inches long, and 20 inches deep. These sleeves are welded to the top of the tank so that only the sleeves open into the air chamber. Over each sleeve there is a detachable cover made of 2-inch cypress in which four equally spaced circular openings have been cut to hold the soil containers.

The tank is filled with water or some non-freezing solution, depending on the temperature which is to be maintained. The tank solution is prevented from coming in contact with the soil containers by the metal sleeves in which

the containers are suspended. However, the sleeves themselves can be filled with water or some other solution in order to facilitate the transference of heat between soil and tank.

The pots holding the soil in which the experimental plants are grown are made of 24-gauge galvanized iron soldered at the seams to make them water-tight. They are 8 inches in diameter at the top, 7 inches in diameter at the bottom, and 18 inches deep. Each soil can has a flange made of  $\frac{1}{2}$ -inch angle iron riveted to its upper rim. This flange not only strengthens the pot, but also supports it when it is suspended in the sleeve of the soil temperature tank, the flange resting on the edge of the opening in the cover.

Each soil container is also provided with a bail which, when turned down, fits snugly against the upper edge of the flange.

In order to maintain the liquid in the soil temperature tanks at a constant temperature, each tank is provided with a cooling coil, a heating unit, and a circulatory system. A perspective view of one of the soil temperature tanks with cover and sleeves removed to show the temperature regulating devices is represented diagrammatically in figure 7.

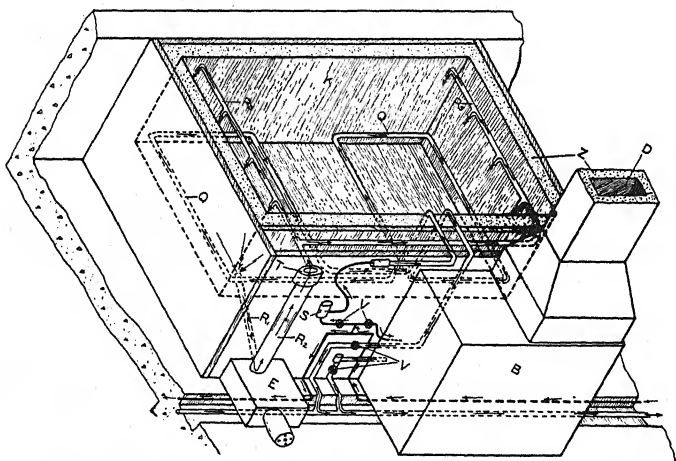


FIG. 7. Perspective view of soil temperature tank with cover and sleeves removed. B, air cooler; D, air duct; E, rotary pump; K, tank; Q, cooling coil; R<sub>1</sub>, outlet pipe from tank to pump; R<sub>2</sub>, R<sub>3</sub>, return pipes from pump to tank; S, solenoid valve; T, immersion heater; V, refrigerant valves; Z, cork insulation.

The cooling coil consists of a 25-foot length of  $\frac{1}{2}$ -inch copper tubing resting on the bottom of the tank (A, fig. 7). The flow of refrigerant through the coil is regulated automatically by a solenoid valve (S, fig. 7), or manually by opening and closing a gas valve placed in the refrigeration line (V, fig. 7).

A uniform temperature throughout the tank is maintained by constantly circulating the solution. This is accomplished by drawing the liquid from

the bottom of the tank through a pipe ( $R_1$ , fig. 7) leading to a motor driven rotary pump (E, fig. 7), and returning it at the top of the tank near each end through 8 spaced outlets in the return pipes ( $R_3$ , fig. 7). The pump and return pipe ( $R_2$ , fig. 7), which lie outside of the compartment, are protected against rapid heat loss or absorption by cork insulation.

Heat is supplied to the solution by a Chromalox Immersion Heater, similar to Model M-175. It is inserted in a T-joint at the upper end of a vertical 2-inch return pipe (T, fig. 7). This heater, rated at 300 Watts, has a blade 9 inches long and is operated by a 110-volt, single phase circuit.

#### REFRIGERATION

All cooling coils are connected to a Frigidaire Model FW6J compressor located inside the greenhouse (A, figs. 2, 4, and 6). The compressor has a water-cooled condenser, through which water is circulated by means of a motor-driven rotary pump (G, fig. 5). The water is cooled by being run through a cooling tower outside of the greenhouse, and is then recirculated through the condenser. A 3-horsepower motor operating on a 220-volt, 3-phase circuit drives the compressor. Freon is the refrigerant.

The compressor motor is switched on and off by means of a pressure switch operated by changes in the pressure of the refrigerant and acting through a relay. In this manner, the opening of any one of the six solenoid valves, each of which controls the flow of refrigerant through a cooling coil, will cause the compressor to operate. When all solenoid valves are closed, the compressor stops. An excessively high pressure caused by the failure of water to flow through the condenser also breaks the circuit to the compressor motor.

The same pressure switch and relay which automatically start and stop the compressor motor also start and stop simultaneously the pump which circulates water through the condenser and the fan in the cooling tower. An additional circuit is provided, however, by which the water-circulating pump can be put into continuous operation independent of the operation of the compressor. This is done in cold weather to prevent freezing in the water pipes outside of the greenhouse. The cooling tower fan can also be put into continuous operation, or turned off.

#### CONTROL OF TEMPERATURE

Each temperature control unit is equipped with a Brown two-pen recording control thermometer model 6422-830 (C, figs. 3 and 4). These instruments simultaneously record and control the temperature of both the air chamber and soil temperature tank. The thermometer bulbs and capillary tubing are of the mercury expansion type. One bulb of each instrument is

immersed in the solution of the soil temperature tank, the other is suspended below the baffle (L, fig. 6) in the stream of air flowing out of the air chamber. The temperature measured by each bulb is recorded on the seven-day chart of the recording instrument.

The control point for each bulb is set at the desired temperature, which may be the same or different, for the soil temperature tank and the air chamber of the same unit. A three-point mercury switch within the instrument is so wired that a neutral contact is made whenever the temperature is that for which the control is set. When the temperature drops below the control point, a contact is made by the three-point mercury switch which, through a relay, completes the circuit to one of the electric heaters. If the temperature rises above that for which the instrument is set, the three-point mercury switch makes a contact which opens a solenoid valve allowing the refrigerant to expand in one of the cooling coils. The differential in temperature required to throw the 3-point mercury switch to the high or low temperature side of the neutral point is adjustable.

### Performance

Extensive tests to determine the performance of this equipment under the extremes of outside temperatures to which it has been exposed have not been made. The equipment, however, has been operated extensively for the purpose for which it was designated: the growing of plants at controlled temperatures. Thus from January 11 to March 10, 1937, the growth chambers were operated at air and tank temperatures of 40°, 50°, and 60° F. Outside air temperatures ranged from a low of -6° to a high of 68° F. during this period. During mild, clear days, the air temperature in the 40° compartment would rise to a maximum of 50° F. at mid-day. Except during this brief period, air temperature did not vary from the setting of 40° by more than  $\pm 2^\circ$  F., or from settings of 50° and 60° by more than  $\pm 2.5^\circ$  F. At no time did the temperature of the soil temperature tanks vary from the set temperature by more than 1° F. in any of the compartments.

From March 18 to May 13, 1937, the growth chambers were operated at air and tank temperatures of 60°, 70°, and 80° F. During this period, outside temperatures ranged from 23° to 86° F. No difficulty was experienced in maintaining the temperatures for which the controls were set within a maximum range of  $\pm 2.5^\circ$  in the case of air temperatures and 1° in the case of tank temperatures.

From May 31 to July 26, 1937, the growth chambers were maintained at air and tank temperatures of 80°, 90°, and 100° F. During this period outside air temperatures ranged from 58° to 96° F. Again air temperatures within the compartments were held within a range of  $\pm 2.5^\circ$  from the set temperature, and tank temperatures within a range of  $\pm 1^\circ$  F.

The temperature ranges given above represent extreme variations which did not occur during the greater part of the time in which the growth chambers were in operation. Furthermore, except in the case of air temperature in the 40° compartment on mild clear days, the variations above and below the set temperature were equal.

From July 28 to September 20, 1936, all three soil temperature tanks were maintained at 70° F. while air temperatures were held at 70°, 85°, and 100° respectively. Variations from set temperatures during this period were no greater than previously described. Outside air temperatures ranged from a minimum of 41° F. to a maximum of 98° F. during this period.

From September 24 to November 19, 1937, the tanks were again maintained at 70° F. in all three growth chambers, while air temperature was also maintained at 70° in one compartment. In another growth chamber an air temperature of 80° was maintained during the day and one of 60° during the night. In the third growth chamber a 90° day and a 50° night air temperature were maintained. Approximately one-half hour was required to change the air temperature from the day to night or night to day setting and with the exception of this brief period, air and tank temperatures were held constant within the ranges previously given. The diurnal changes in the setting of the controls had to be made by hand, but this was a simple operation. Outside air temperatures ranged from a minimum of 18° to a maximum of 94° F. during this period.

From November 21, 1937, to January 15, 1938, the growth chambers were again operated at air and tank temperatures of 60°, 70°, and 80° F. with no greater variations from control settings than reported for previous periods. The range of outside air temperatures during this period was from 5° to 60° F.

In order to test the cooling capacity of the soil temperature tanks, one was lowered to a temperature of -10° F. and held at this level from 4 to 5 P.M. The average outside temperature on this date was 46° F.

One growth chamber was maintained at an air temperature of 60° F. from 1 to 3 P.M., May 20, 1937, a clear afternoon, with an outside wet bulb temperature of 75° and a dry bulb temperature of 88° F. No water was run over the roof of the growth chamber during this test period.

Since at no time has it been necessary to use more than one-half of the heating capacity of the air conditioning unit (two heaters controlled by separate switches are provided in each), much higher temperatures than have been applied so far can undoubtedly be attained.

During the first year of operation, mechanical failures of the equipment have been infrequent and of a minor nature. Because of the use made of standard air-conditioning and refrigeration equipment, repair parts are

readily obtained, and necessary repairs and adjustments can be made by the local refrigerator service department.

BUREAU OF PLANT INDUSTRY

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## GLYCINE IN THE NUTRITION OF EXCISED TOMATO ROOTS

PHILIP R. WHITE

(WITH TEN FIGURES)

Five years ago it was unequivocally demonstrated for the first time that an isolated plant organ, the root, could be grown in culture for potentially unlimited periods of time (8). An environmental complex capable of supporting normal growth for such periods was outlined which included only one factor recognized as being of unknown constitution. That factor was an extract of dried brewers' yeast representing 1/10,000 of the total mass of the nutrient. Since this one unknown made interpretations of any experiments utilizing the complex of uncertain validity, much effort has been expended since then aiming at the elimination of this factor. Analysis showed the effective material to be soluble in 85 per cent. alcohol, insoluble in ether, and separable by extraction with 100 per cent. alcohol into two fractions both of which were essential for normal growth (10). The fraction insoluble in 100 per cent. alcohol was shown to be replaceable by a mixture of 9 amino acids (11). This demonstration was obtained before the nature of the 100 per cent. alcohol soluble material, now known to be thiamin or its precursors, was known (1, 4, 12). A nutrient containing thiamin and amino acids was still inferior to the original yeast extract medium. It was shown to be improved by addition of a complex mixture of accessory salts. Analysis of the accessory salt mixture showed most of the ingredients to be either inert or detrimental, and by elimination of all but four (iodine, manganese, zinc, and boron), the nutrient was improved to the point where growth equal to that in a yeast extract medium could be obtained (13). A completely known nutrient for the maintenance of normal growth of excised tomato roots was thus made available.

The nutrient so developed was, however, rather complicated, consisting of C. P. grade sucrose, 9 amino acids, thiamin, 10 salts of C. P. grade, and redistilled water. The last step in its development had involved an analysis of the earlier accessory salt mixture and had resulted in considerable simplification. It was suggested at that time (13) that a similar analysis of the amino acid mixture was desirable. This was particularly so since the amino acid mixture had been developed at a time when the nutrient still included an unknown factor, the material soluble in 100 per cent. alcohol, now known to be thiamin. This paper presents the results of an investigation of the amino acid mixture previously used, in the presence of adequate amounts of thiamin and of a satisfactory supply of accessory salts. The materials and methods were those already outlined elsewhere.



## Experimentation

An attempt was first made to determine if any single acid might be dispensed with. Doubt had existed from the first as to the really essential character of proline, valine, and serine. An experimental series was set up in which each of the 9 amino acids was omitted in turn from an otherwise complete nutrient. The result, shown in figure 1, was quite unexpected. Omission

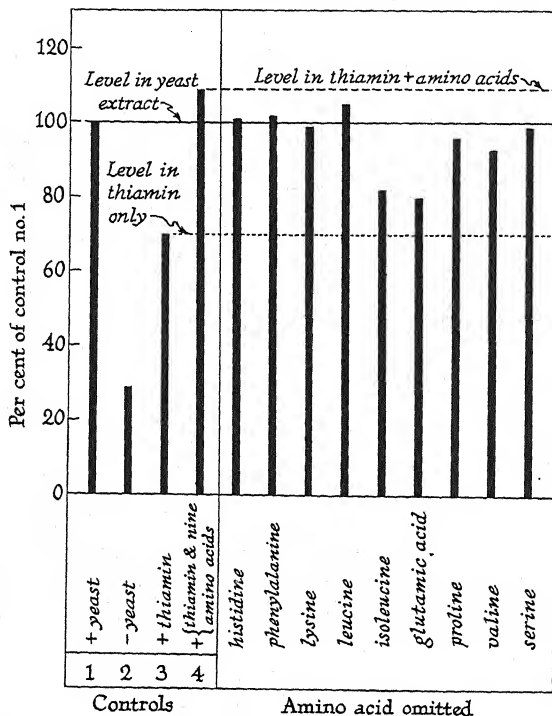


FIG. 1. Histogram showing the effect of omitting single amino acids from an otherwise complete nutrient containing standard salts, accessory salts, sugar, thiamin, 8 of the earlier 9 amino acids, and redistilled water. Only in the cases of isoleucine and glutamic acid is the effect sufficient to be of possible significance.

sion of histidine, phenylalanine, lysine, leucine, proline, valine, or serine in no case resulted in significant diminution in growth rate. Each was apparently unessential when the other 8 acids were all present. Growth in the absence of isoleucine was about 17 per cent. less than in its presence, and in the absence of glutamic acid about 20 per cent. less. But differences of less than 30 per cent. have in past work not been considered of certain significance. Even isoleucine and glutamic acid were thus of doubtful importance.

Moreover, omission of histidine and phenylalanine, histidine and lysine, histidine and leucine, histidine and isoleucine, and of lysine and leucine

likewise gave results which in no case differed significantly from the controls. It is particularly noteworthy that, although omission of isoleucine alone had

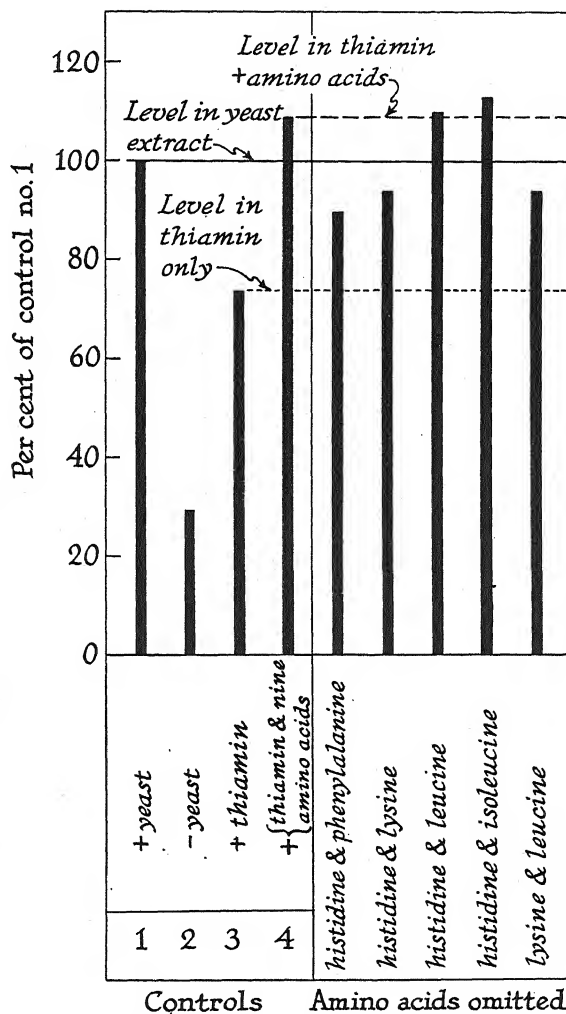


FIG. 2. Effect of omitting pairs of amino acids from an otherwise complete nutrient. In no case is the effect sufficient to be of significance.

earlier given a possibly significant decrease (17 per cent.), when both isoleucine and histidine were omitted the results were 13 per cent. better than the control. The earlier decrease was attributable to random variation due to unknown causes and was not significant. No one of these amino acids and none of the pairs tested was indispensable. Yet growth in the absence of all

9 amino acids was regularly between 30 per cent. and 60 per cent. less than in their presence. "Amino acid" seemed to be essential for normal growth but not any particular amino acid.

Since no single amino acid of the original group could be shown to be indispensable, the suspicion arose that the amino acid requirement of these roots must be far simpler than the earlier work had indicated. It may be recalled that one chart used two years ago (11, p. 794, fig. 1) showed that glycine alone would support growth almost equal to the control, for one passage. But when tested over a second passage, in the absence of thiamin, the growth rates dropped to practically nil (fig. 3). This, together with the

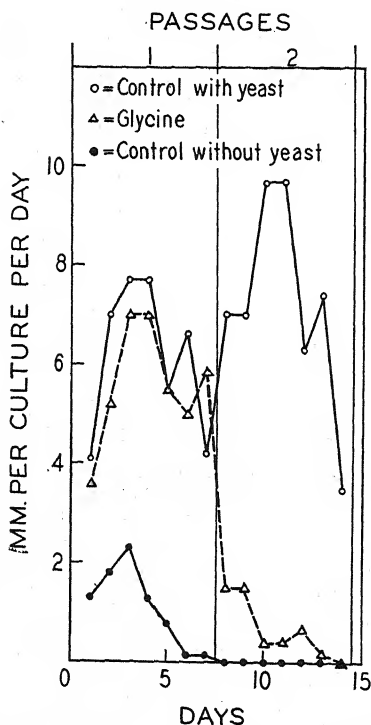


FIG. 3. Graph showing the effect of adding 5 p.p.m. of glycine to a basic nutrient containing standard salts, accessory salts, and sugar, recorded over two passages. While growth in the first passage was almost equal to that in a yeast extract nutrient, in the second passage the growth rate dropped almost to zero.

fact that addition of glycine to the 9-amino-acid mixture under consideration did not improve it, led at that time to the abandonment of the study of glycine. Now it appears that in these experiments the limiting factor was not glycine, but thiamin. It seemed desirable, therefore, to re-investigate the effects of glycine, this time in the presence of thiamin.

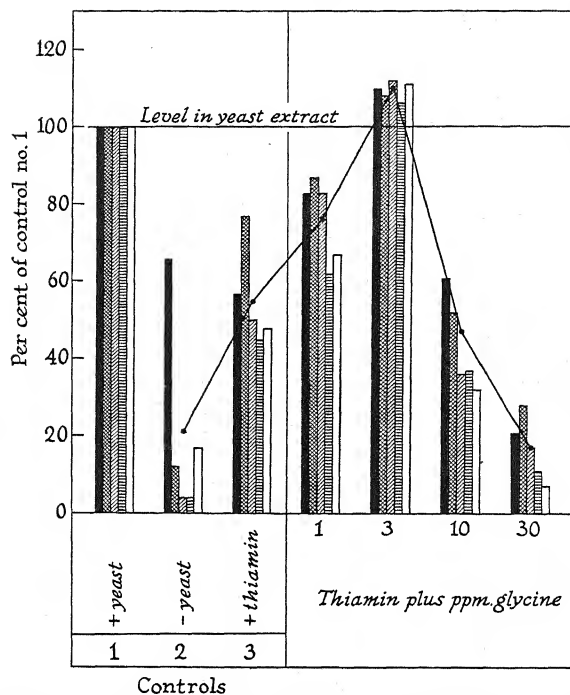


FIG. 4. Effect of adding 1, 3, 10, and 30 p.p.m. of glycine to a nutrient like that used in figure 3, but with the addition of 0.1 p.p.m. of thiamin. With 3 p.p.m. glycine, the increment exceeded that in a yeast extract nutrient in every one of the 5 passages.

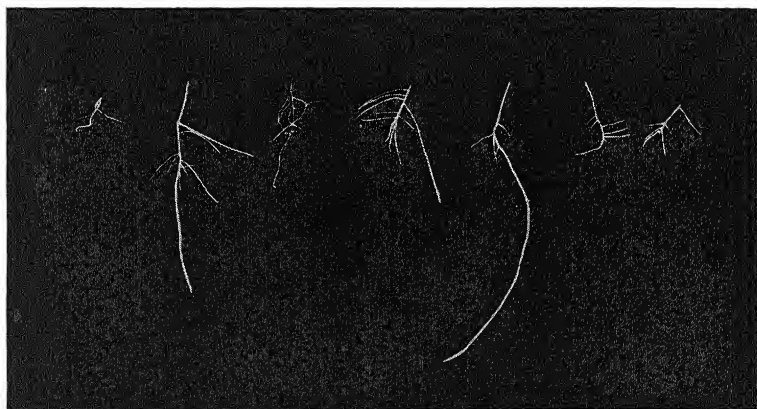


FIG. 5. Photograph of roots grown in nutrients containing in addition to  $H_2O$ , standard and accessory salts, and sugar, the following supplementary materials, reading from left to right: none (negative control); yeast extract (positive control); thiamin only; and thiamin plus 1, 3, 10, and 30 p.p.m. glycine. These roots were from passage 5 (the open columns) of figure 4. Note that roots grown in a nutrient supplemented by thiamin only were thin and obviously in poor condition. (Photograph by J. A. CARLILE.)

A concentration series in which yeast extract was replaced by accessory salts, 0.1 p.p.m. thiamin, and 1, 3, 10, or 30 p.p.m. glycine gave the results shown in figures 4 and 5. While 10 p.p.m. and 30 p.p.m. glycine were evidently injurious and 1 p.p.m. was inadequate to support normal growth, the root growth in a solution containing 3 p.p.m. glycine was superior to that in the control nutrient containing yeast extract during every one of five consecutive passages. A second concentration series fixed the optimum concentration at between 2 and 4 parts per million (fig. 6). This laboratory's

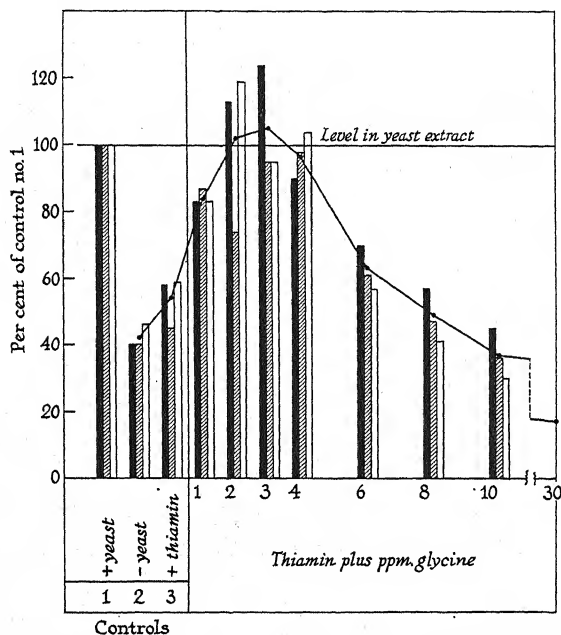


FIG. 6. Effect of adding 1, 2, 3, 4, 6, 8, and 10 p.p.m. glycine to a nutrient like that used in figure 3, but with the addition of 0.1 p.p.m. of thiamin. The optimum lies between 2 and 4 p.p.m. glycine (*cf.* fig. 4).

6-year-old stock of tomato roots was subsequently transferred to a nutrient in which the organic constituents of yeast extract were replaced by 0.1 p.p.m. thiamin plus 3 p.p.m. glycine, and grown for 11 weeks. The average growth rate of these cultures in this 11-week period was excellent and was equal to that in any previous single 11-week period except the corresponding period a year before (June-July-August, 1933 = 2.9, 1934 = 7.2, 1935 = 8.0, 1936 = 8.5, 1937 = 10.0, 1938 = 9.7 mm. per culture per day).

It is clear then, that the amino acid requirements of excised tomato roots can be supplied by the single amino acid, glycine ( $\alpha$ -amino-acetic acid) at a concentration of 3 p.p.m. or  $4 \times 10^{-5}$  M. The next more complex amino acid,  $\alpha$ -amino-propionic acid (alanine), which in the  $\beta$  form has been reported to

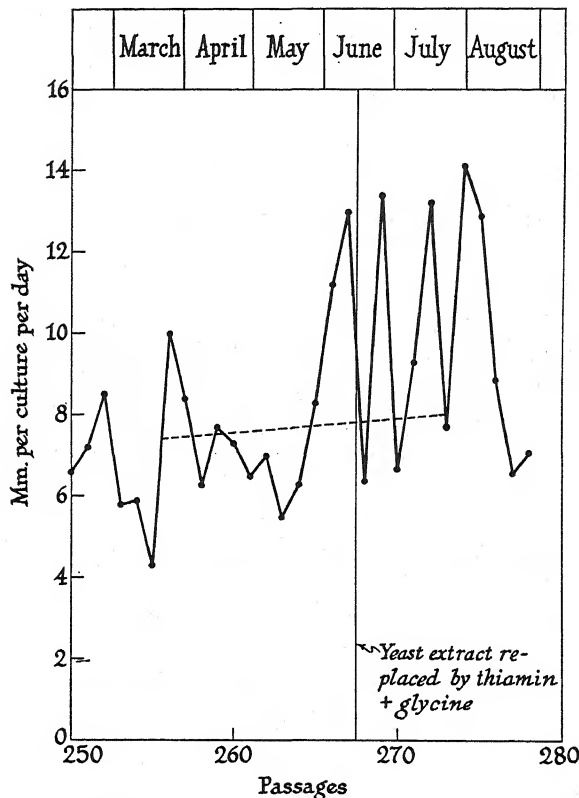


FIG. 7. Mean growth rates of excised tomato roots through 18 passages in a yeast extract nutrient followed by 11 passages in a glycine-thiamin nutrient. The dotted line indicates approximately the seasonal increase to be expected in a yeast nutrient over this period of the year (7).

be a growth requirement for some bacteria, proved extremely toxic at equivalent concentrations. Isoleucine ( $\beta$ -methyl- $\alpha$ -amino-valeric acid) was somewhat less toxic. Glutamic acid ( $\alpha$ -amino-glutaric acid), aspartic acid ( $\alpha$ -amino-succinic acid), and lysine ( $\alpha$ - $\epsilon$ -diamino-caproic acid) showed neither a stimulating nor a depressing effect at similar concentrations. This behavior of aspartic acid is of particular interest in view of the reported rôle of its acid amide, asparagine, as the usual amino nitrogen transport substance in plant nutrition. This evidence makes such a rôle seem extremely doubtful in the case of the tomato. Although not all of the amino acids of the original list of 9 have been tested singly, it appears that the more complex forms may not be satisfactorily utilized by tomato roots for transformation into dissimilar amino acids, and that only the most simple form ( $\alpha$ -amino-acetic acid) is available for this purpose.

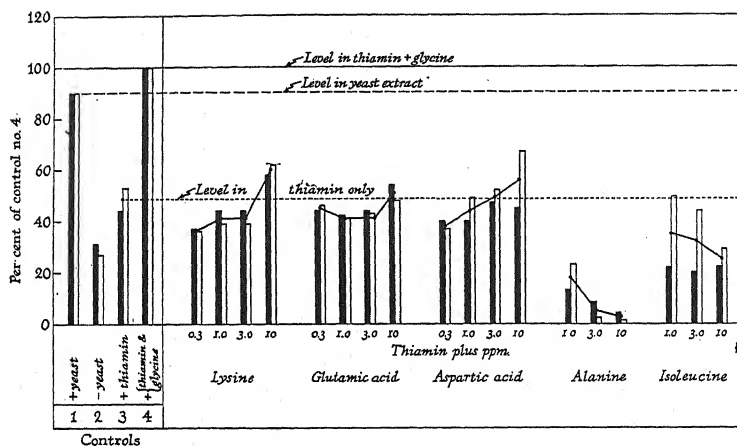


FIG. 8. Effect of adding various concentrations of lysine, glutamic acid, aspartic acid, alanine, and isoleucine to a thiamin nutrient. In no case is there a significant increase.

Amino-acetic acid has two important groups: the amino group and the carboxyl group. These experiments have shown that the amino group is not effective if attached to the propionic, valeric, caproic, succinic, or glutaric radicals. To determine if the carboxyl group would be equally effective if attached to other cations, acetic acid, citric acid, and malic acid were tested at molar concentrations equivalent to those used with glycine. The former

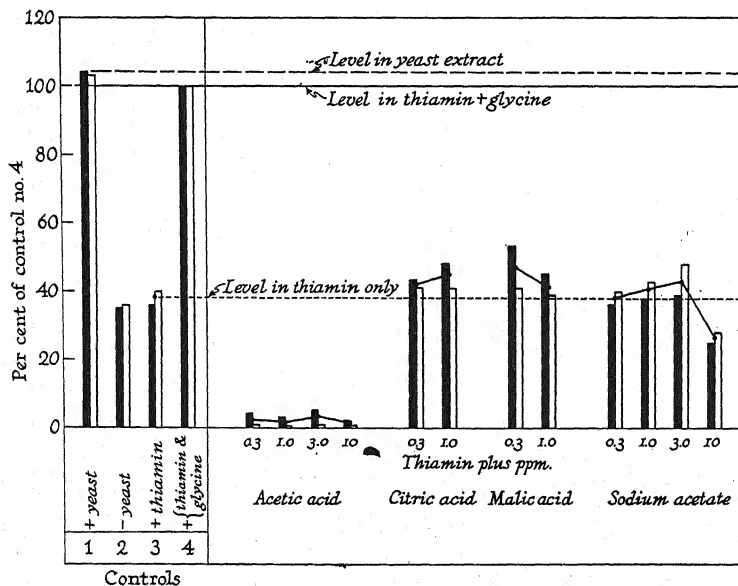


FIG. 9. Effect of adding acetic, citric, and malic acids, and sodium acetate to a thiamin nutrient in concentrations approximating those previously used for glycine.

was markedly toxic, while the latter two were without significant effect (fig. 9). The acetate portion was likewise shown to be without effect when supplied as sodium acetate. The effectiveness of glycine is, then, not owing merely to its supplying amino nitrogen,  $\alpha$ -amino groups, carboxyl groups, or acetate. It functions neither as an organic acid nor merely as an amino acid. Glycine appears to be a growth-promoting substance *per se* rather than because of any of its components.

### Discussion

Glycine alone is, then, capable of supplying all the amino-nitrogen required for growth of excised tomato roots other than that which they may be able to synthesize from inorganic nitrate. The validity of this conclusion seems unimpeachable. Yet glycine represents, according to MEISENHEIMER (3), only 0.5 per cent. of the entire amino acid content of yeast, and the amount of glycine present in the optimal concentration of yeast extract, calculated on this basis, is only 0.03 mg. per liter. This is 1/100 of the glycine concentration optimal for growth and would be quite inadequate in itself to support the increments observed in a yeast extract medium. It is clear that yeast extract can not owe any considerable part of its effectiveness to its glycine content, but to the particular balance of other substances present. Glycine is entirely wanting in zein, hordein, gliadin, vicillin, and some other storage proteins (OSBORNE and associates). The demonstration of its capacity to supply all of the amino acid required by excised tomato roots can not, therefore, be interpreted as in any way indicative of the nature of the substance or substances normally supplied by the green tissues of the intact tomato plant to its roots. We are apparently dealing here merely with a workable substitution.

However remote this result may be from representing the conditions in the intact plant, it is, nevertheless, a step forward in our efforts to establish a completely known nutrient for the cultivation of isolated roots since it permits at one stroke the elimination of 8 organic ingredients which might have presented difficulties when it came to removing the last traces of impurities from the nutrient. The organic constituents of the nutrient are reduced to three: sucrose, thiamin, and glycine. In our present state of knowledge, these or their equivalents can not be omitted, although ROBBINS and BARTLEY (5) and BONNER (2) have shown that in some cases the thiamin can be replaced by the simpler thiazole or pyrimidine, or by a mixture of both. ROBBINS and SCHMIDT (6, 7) have presented evidence that vitamin B<sub>6</sub> may benefit growth of excised tomato roots in a solution containing nutrient salts, sucrose, and thiamin. The optimal concentration of vitamin B<sub>6</sub> appears to be about 1 mg. per liter (50  $\gamma$  per flask containing 50 ml. of nutrient). The growth increase produced by this addition is reported to be about 100 per cent.



The present paper shows that about the same increase is obtained when 3 mg. of glycine are added in place of 1 mg. vitamin B<sub>6</sub>. While differences in method preclude any direct comparison between the results of ROBBINS and SCHMIDT and those reported here, it is interesting to note that glycine and vitamin B<sub>6</sub> appear to have, weight for weight, growth-promoting properties of about the same magnitude. Experiments to test the effects of these two substances in the presence of one another are now in progress. Since both thiamin and glycine are required in extremely small amounts and are both synthetic products, only the sucrose remains as a possible source of unknowns. Repurification of these crystalline ingredients represents, it may be hoped, the last step in the establishment of a completely known nutrient for the cultivation of excised tomato roots.

Caution must be employed, however, in extending these conclusions to species other than tomato. This laboratory maintains root cultures of a number of other plants. Naturally, the glycine-thiamin nutrient was tried on them. Most species do well. But, as figure 10 shows, such a nutrient

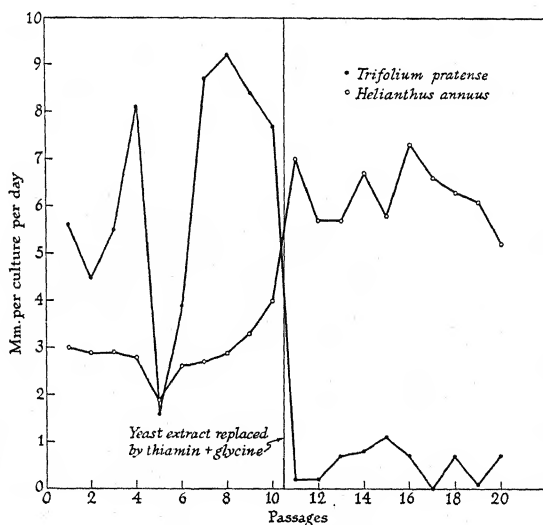


FIG. 10. Behavior of roots of *Trifolium pratense* L. and *Helianthus annuus* L. in nutrients in which yeast extract was replaced by thiamin, glycine, and accessory salts.

does not support growth of clover, while sunflower roots do much better in it than in a yeast nutrient. Roots of legumes and of composites behave quite differently. These differences present in themselves vast fields for research.

### Summary

Analysis of the 9 amino-acid mixture earlier developed as a constituent of

the nutrient for cultivation of excised tomato roots showed that no single amino acid and none of a number of pairs of acids was indispensable. Glycine alone proved capable of replacing the entire group. Neither the  $\alpha$ -amino group, the carboxyl group, nor the acetate radical was effective when supplied in any of several related compounds. Since glycine is not present in many storage proteins and is present only in small amount in yeast, no conclusions as to the form in which amino acid may be supplied to roots in the intact plant can be drawn from this result. A completely known and relatively simple nutrient is now available for the cultivation of excised tomato roots. This nutrient as now constituted contains 20 gm. sucrose, 100 mg. calcium nitrate, 35 mg. magnesium sulphate, 80 mg. potassium nitrate, 65 mg. potassium chloride, 12.5 mg. potassium acid phosphate, 0.75 mg. potassium iodide, 2.5 mg. ferric sulphate, 4.4 mg. manganous sulphate, 1.5 mg. zinc sulphate, 1.6 mg. boric acid, 3 mg. glycine and 0.5 mg. thiamin per liter. This nutrient is capable of supporting continued and probably unlimited growth of tomato roots. Further study may perhaps still further simplify this solution.

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# EFFECTS OF CATIONS AND ANIONS ON PROTOPLASMIC ELASTICITY<sup>1</sup>

HENRY T. NORTHEN AND REBECCA T. NORTHEN

## Introduction

It is generally believed that one of the chief rôles of cations is to maintain the protoplasmic colloids in the proper state of dispersion and aggregation. Many authors have compared protoplasm to non-living colloidal systems and assume that the electric charge of the colloidal particles is of first significance in preventing the precipitation of the particles.

If the charge on the protoplasmic colloids is of prime importance in maintaining the stability of the system, the valence of introduced cations should largely determine the effects of such ions in dispersing or aggregating the colloids. A greater dispersion of the colloids should result in a decrease in protoplasmic consistency and an aggregation should result in an increase. As yet, there is no general agreement as to the effects of cations on protoplasmic consistency. SEIFRIZ (7) states that "the effect of sodium and of calcium on protoplasm is a controversial subject, though the consensus of opinion appears to indicate that, in general, sodium disperses and calcium aggregates." On the other hand, HEILBRUNN (1) maintains that sodium and potassium increase the viscosity of the main mass of protoplasm whereas calcium and magnesium decrease the viscosity. In *Amoeba*, however, the cortical protoplasm does not respond to cations as does the endoplasm (1). In the cortical protoplasm HEILBRUNN reports that sodium, potassium, and magnesium cause liquefaction whereas calcium causes a pronounced stiffening.

Previous investigations carried out in this laboratory indicate that the protoplasm in which the chloroplasts of *Spirogyra* are embedded is not a simple colloidal system such as the physical chemist studies. For example, NORTHEN (2) has noted that when protoplasm in cells of *Zygnema* and *Spirogyra* is cooled the consistency reaches a maximum as rapidly as temperature equilibrium is attained, whereas in non-living hydrophilic colloidal systems (*e.g.*, gelatin solution) the consistency increases during a long period after temperature equilibrium has been reached. One assumption of NORTHEN, however, should be corrected. At the time, NORTHEN stated that it was probable that the displacement of the chloroplasts by centrifugal accelerations was limited by the ease with which the chloroplasts could bend rather than by the resistance of the cytoplasm. Observations on the appearance of cells following centrifugation clearly indicate that the elasticity of the cytoplasm is the limiting factor rather than the ease with which the chloro-

<sup>1</sup> Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 171.

plasts are bent. Following centrifugation the main mass of the chloroplasts are aggregated at the centrifugal end of the cell but the ends of the chloroplasts extend back (have "unbent") from the aggregated mass (see illustrations in HEILBRUNN 1, page 76, and in NORTHEEN 2, page 195). The extended portions indicate that the cytoplasm is impeding their motion, so much so that they are straightened out.

NORTHEEN (3) has found that the protoplasm in which the chloroplasts of *Spirogyra* are embedded is an elastic fluid and has deduced (6) that protoplasm has a net-like ultramicroscopic structure. Supposedly the network results from the combination of proteins with lipoids and carbohydrates. It is probable that many inorganic ions are tied up to the network or occupy key positions in the network such as magnesium does in the chlorophyll molecule. The network is a labile structure rather than a stable one. The structure is readily altered by injury (4), dilute solutions of anesthetics (5), and by temperature (6). We doubt if any known non-living system can respond to the above stimulants as does protoplasm. The responses of protoplasm are unique and hence it is reasonable to believe that protoplasmic responses to cations and anions will also be unique.

According to the preceding concept the protoplasmic system is semi-stable because molecules have definite places in the network and because they are not in suspension. For example, protein molecules in the network can aggregate only when they are liberated from the other constituents of the network (go into colloidal suspension), and of course then only when other requisites of aggregation are favorable. In a structure such as postulated, the chemical properties of an ion will be of more importance in altering the structure than will be the valence of the ion. The data presented will show that the valence of introduced cations or anions has little effect on the structure of the protoplasm.

A discussion of the effects of cations on protoplasm should include mention of ion antagonism. It is common knowledge that the toxic effects of the sodium ion can be counteracted by the calcium ion. According to SEIFRIZ (7) the univalent sodium ion liquefies protoplasm and the divalent calcium ion aggregates, but when the two are mixed together the protoplasm remains normal. Assuming that Na and Ca act as SEIFRIZ states, that type of antagonism can be explained on the basis of charge carried by the ions. The antagonism of the divalent magnesium ion by the divalent calcium ion or the divalent strontium ion (8) cannot be explained, however, on such a basis. The data presented will show that the magnesium ion tightens the protoplasmic structure (increases elasticity) and that both the calcium and strontium ions loosen the network (decrease elasticity). The calcium ion and to a lesser degree the strontium inhibit the effect of the magnesium ion. One cannot, however, always predict from a study of single cations what the effect

will be when two ions are allowed to act at the same time. For example, it will be demonstrated that both magnesium and cadmium when acting singly cause a decided increase in elasticity but that when cadmium and magnesium act together they produce a decrease in elasticity.

### Method

The method is based on the equation:  $V = k(c - c_0)$ , which approximately governs the rate with which the chloroplasts in cells of *Spirogyra* move in response to different centrifugal accelerations (3). In the above equation  $V$  is the velocity of chloroplastic movement,  $k$  a constant,  $c$  the centrifugal acceleration used, and  $c_0$  is the initial starting centrifugal acceleration at which, or below which, the chloroplasts will not move regardless of how long the acceleration is allowed to act. To understand the meaning of the above equation, imagine a test-tube filled with an elastic fluid, a fluid in which long intermeshed molecules form a network. If a ball is now introduced, it will not sink because the net holds it up. Next centrifuge the tube. With low accelerations the ball will not move, regardless of how long the tube is centrifuged, because the low centrifugal acceleration acting on the ball does not create a sufficient force to shear apart the interlaced molecules. If the acceleration is increased so as to produce the necessary shearing force, the ball will move. The maximum acceleration which is not sufficient to shear apart the interlaced molecules has been designated as  $c_0$ . With accelerations greater than  $c_0$  the ball will move. Likewise, in cells of *Spirogyra* the network must be sheared apart before the chloroplasts can move through the cytoplasm.

Variability characterizes organisms. In a population of *Spirogyra*  $c_0$  is not the same for all of the filaments. Hence when a number of filaments are centrifuged with an acceleration of  $680 \times$  gravity, the chloroplasts will be displaced in cells of filaments whose value of  $c_0$  is less than  $680 \times$  gravity and will not be displaced in cells in which the value of  $c_0$  is equal to or greater than  $680 \times$  gravity because  $V = k(680 - 680) = 0$  or  $V = k(680 - > 680) = < 0$ . Any treatment which loosens the structural network will decrease the value of  $c_0$ , and accordingly when treated filaments are centrifuged with an acceleration of  $680 \times$  gravity the chloroplasts will be displaced in more cells than in untreated ones. On the other hand any treatment which tightens the structure will increase the value of  $c_0$  and concomitantly decrease the percentage of cells in which the chloroplasts are displaced (moved to centrifugal end). A decrease in  $c_0$  has been termed a decrease in elasticity, whereas an increase in  $c_0$  has been interpreted as an increase in elasticity.

Filaments of *Spirogyra* were immersed for one- and two-hour intervals in solutions of various cations and anions, and in some experiments in solutions of mixed cations. To minimize osmotic effects approximately isosmotic solutions have been used. If the molecules dissociated into two ions  $0.07\text{ M}$

solutions were used; if into three ions 0.05 M solutions were used; and if into four ions 0.035 M solutions were used. After the filaments had been in the solutions for the desired time they were placed between strips of cotton soaked in the appropriate solution and centrifuged with an acceleration of  $680 \times$  gravity for thirty seconds. Following centrifugation the percentages of filaments in the cells of which the chloroplasts had been displaced were

TABLE I

EFFECTS OF UNIVALENT AND DIVALENT CATIONS ON PROTOPLASMIC ELASTICITY

GROUP I	GROUP II
Hydrogen*	
D Lithium 75 per cent.—1 hour 86 per cent.—2 hours	I Beryllium 6 per cent.—1 hour 1 per cent.—2 hours
I Sodium 32 per cent.—1 hour 20 per cent.—2 hours	I Magnesium 8 per cent.—1 hour 6 per cent.—2 hours
D-I Potassium 85 per cent.—1 hour 16 per cent.—2 hours	D Calcium 93 per cent.—1 hour 61 per cent.—2 hours
I Copper 0 per cent.—1 hour 0 per cent.—2 hours	I Zinc 0 per cent.—1 hour 0 per cent.—2 hours
Rubidium*	D Strontium 80 per cent.—1 hour 71 per cent.—2 hours
I Silver 0 per cent.—1 hour 0 per cent.—2 hours	I Cadmium 0 per cent.—1 hour 0 per cent.—2 hours
O-D Caesium 46 per cent.—1 hour 44 per cent.—2 hours	D Barium 100 per cent.—1 hour 100 per cent.—2 hours
Gold*	I Mercury 0 per cent.—1 hour 0 per cent.—2 hours
Distilled Water 52 per cent.—1 hour 30 per cent.—2 hours	Tap Water 73 per cent.—1 hour 73 per cent.—2 hours
	O = little or no effect I = elasticity increased D = elasticity decreased

\* Ion not used.

determined. For each experimental group between 400 and 500 filaments were examined.

### Data and discussion

The effects of univalent and divalent cations on the elasticity of protoplasm in *Spirogyra* are recorded in table I. The sulphate salt of beryllium and the nitrate salt of silver were used. In all other instances the chlorides were used. In the table the elements are arranged as in the periodic table. Below each element the percentages of filaments in the cells of which the chloroplasts were displaced by centrifugation after one hour's immersion and two hours' immersion are recorded. The letter O above an ion indicates that when compared to distilled water (the solvent) that ion had little effect. The letter D indicates that the ion decreased protoplasmic elasticity and the letter I indicates that the ion increased the elasticity. It should be remembered that an increase in elasticity indicates a tightening of the network and a decrease in elasticity indicates a loosening of the network.

It will be noted, table I, that the valence of the ion has little effect in determining whether the elasticity will be decreased or increased. For example, the monovalent lithium ion decreases elasticity whereas the monovalent sodium ion increases it.

In group II it will be noted that the ions of chemically similar elements act approximately alike. Chemically beryllium, magnesium, zinc, cadmium, and mercury are related and their ions cause an increase in the elasticity of protoplasm. Chemically calcium, strontium, and barium are closely related and their ions act alike in decreasing protoplasmic elasticity. Hence it appears that changes in protoplasmic structure are correlated with the chemical nature of the divalent cation, and accordingly the effects of the divalent cations on the stability of the protoplasm seems to be determined by chemical properties rather than by such properties as valence.

Although in group I lithium, sodium, potassium, and caesium are related, they do not affect protoplasm in a like manner. After two hours' immersion the very closely related sodium and potassium ions cause a slight decrease in elasticity. (Differences of less than 8 per cent. are not considered significant because of the variability of the samples.) The caesium ion causes a slight decrease in elasticity whereas the lithium ion causes a pronounced decrease. We do not know why caesium should act differently from sodium and potassium. Typically the first member of a family has many properties which are not shared by subsequent members. Perhaps some of these unique properties explain the liquefying effect of the lithium ion. Chemically copper is related to silver and has much in common with mercury and their ions have a similar action on protoplasmic structure.

Most cations affected protoplasmic structure in one way or another. This



indicates that the cations penetrated, although it is not known how much of each actually did enter the cells. The data suggest, however, that the effects of the cations is chemical, which implies that the cations react in some unknown way with the network constituents. The fact that the ions probably react with network constituents should be considered in studies of permeability and accumulation of ions. Certainly the effects of the cations are not the result of the ions just passing through the cytoplasm. Hence it is likely that some types of cation accumulations are the result of reactions between the cations and protoplasmic constituents.

If one ion decreases protoplasmic elasticity and another increases elasticity, it might be assumed that when the two ions act together the protoplasmic structure will remain approximately normal. That such is not always true, however, is shown by the data recorded in table II, in which experi-

TABLE II  
EFFECTS OF CATION COMBINATIONS ON PROTOPLASMIC ELASTICITY

SOLUTION	PERCENTAGES OF FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED AFTER IMMERSIONS OF	
	ONE HOUR	TWO HOURS
	%	%
Tap water .....	49	49
Distilled water .....	38	25
0.05 M $MgCl_2$ .....	4	2
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.07 M $BeSO_4$ .....	13	3
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $CaCl_2$ .....	31	28
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $ZnCl_2$ .....	0	0
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $SrCl_2$ .....	10	4
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $CdCl_2$ .....	37	57
95 ml. 0.05 M $MgCl_2$ + 5 ml. 0.05 M $BaCl_2$ .....	90	80
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.07 M $BeSO_4$ .....	12	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $CaCl_2$ .....	27	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $ZnCl_2$ .....	0	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $SrCl_2$ .....	17	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $CdCl_2$ .....	36	
90 ml. 0.05 M $MgCl_2$ + 10 ml. 0.05 M $BaCl_2$ .....	77	

ment filaments of *Spirogyra* were immersed in a solution containing magnesium chloride plus some salt of another divalent cation.

Table II demonstrates that the magnesium ion can be decidedly antagonized by the barium, cadmium, and calcium ions. Such results are of especial interest because both the cadmium and magnesium ions by themselves cause an increase in elasticity. In proportions of 9 to 1 the strontium ion exhibits a slight antagonistic effect after one hour's immersion.

The data in table I indicate that magnesium, through chemical changes in

the protoplasmic network, causes an increase in the elasticity of the protoplasm, and the data in table II indicate that the calcium ion can antagonize this effect when the two ions are acting at the same time. The data recorded below demonstrate, however, that once the protoplasmic structure has been decidedly altered by the magnesium ion it cannot be brought back to normal by the calcium ion. In this experiment filaments were immersed for one hour in 0.05 M  $\text{MgCl}_2$ . Next they were transferred to 0.05 M  $\text{CaCl}_2$  solution where they were allowed to remain for intervals of one and two hours before centrifugation.

TREATMENT	PERCENTAGE OF FILAMENTS IN THE CELLS OF WHICH THE CHLOROPLASTS WERE DISPLACED	
	%	
$\text{MgCl}_2$ for one hour .....	8	
$\text{CaCl}_2$ for one hour .....	52	
$\text{MgCl}_2$ for one hour— $\text{CaCl}_2$ for one hour .....	5	
$\text{MgCl}_2$ for one hour— $\text{CaCl}_2$ for two hours .....	2	

The effects of the potassium salts of various anions on protoplasmic elasticity are recorded below. Underneath each ion are recorded the percentages of filaments in the cells of which the chloroplasts were displaced by centrifugation. The figures in parentheses are the percentages of filaments in which the chloroplasts were displaced after an immersion of one hour and the figures which are not in parentheses are the percentages after an immersion of two hours.

$\text{CrO}_4^{=}$ ; $\text{F}^-$ ; $\text{PO}_4^{=}$ ; $\text{SO}_4^{=}$ ; $\text{Br}^-$ ; $\text{I}^-$ ; $\text{Cl}^-$ ; Dist. $\text{H}_2\text{O}$ ; $\text{NO}_3^-$ ; Tap $\text{H}_2\text{O}$ ; $\text{AsO}_4^{=}$											
(22%) (62%) (23%) (33%) (35%) (40%) (26%) (38%) (80%) (43%)											
6% 9% 16% 20% 23% 27% 28% 30% 50% 51% 57%											

When compared with distilled water it will be noted that the nitrate and arsenate ions decrease protoplasmic elasticity. The closely related bromide, chloride, and iodide ions have little effect on elasticity. After two hours' immersion the sulphate, phosphate, fluoride, and chromate ions cause an increase in protoplasmic elasticity. It will be noted that the charge carried by the ions does not determine whether the elasticity will be increased or decreased.

The studies of the effects of cations and anions lend more support to an ultramicroscopic network structure for protoplasm than to a suspension of hydrophobic and hydrophilic colloids whose stability is determined in part by the charge of the suspended particles. An explanation of how the cations and anions affect protoplasmic structure cannot be given at the present time. It should be remembered, however, that there are metallo-organic compounds in cells. In the postulated network, ions may occupy key positions. The

substitution or removal of these ions by others cannot but have an effect on protoplasmic structure and biological processes (*e.g.*, the substitution of copper for magnesium alters the properties of chlorophyll).

### Summary

1. Groups of *Spirogyra* filaments were immersed for one- and two-hour intervals in isosmotic solutions of various cations, anions, and mixed cations. Controls were maintained in distilled and tap water. They were then centrifuged in the appropriate solution with an acceleration of  $680 \times$  gravity for 30 seconds. Comparison of the percentages of filaments in the cells of which the chloroplasts were displaced showed whether the elasticity had been increased or decreased. A decrease in elasticity was interpreted as a loosening of the protoplasmic network, and an increase as a tightening.

2. The univalent cations lithium and caesium decreased protoplasmic elasticity, while sodium and potassium increased it. Of the divalent cations, chemically related calcium, strontium, and barium decreased elasticity, whereas chemically related beryllium, magnesium, zinc, cadmium, and mercury increased it.

3. The closely related anions bromide, chloride, and iodide had little effect on elasticity; the sulphate, phosphate, fluoride, and chromate ions caused an increase, and the arsenate and nitrate ions a decrease.

4. From the above data it was concluded that the effect of an ion in decreasing protoplasmic elasticity is due to its chemical activity rather than to its valence.

5. When filaments were immersed in mixtures of divalent cations, the result could not always be predicted from the behavior of the individual ion. For instance magnesium, which caused an increase in elasticity, was antagonized by barium which caused a decrease. However, magnesium was also antagonized by cadmium which individually acted like magnesium.

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## CHARACTERISTICS OF THE TYROSINASE SYSTEM IN POTATOES WHICH BLACKEN AFTER BOILING<sup>1</sup>

A. FRANK ROSS, W. E. TOTTINGHAM, AND RUDOLPH NAGY

(WITH THREE FIGURES)

The discoloration of white or Irish potatoes after cooking appears to be associated with both instability of the protein and unusual activity of the tyrosinase system (11, 13). MERKENSCHLAGER (7) attributed the abnormality jointly to accumulation of tyrosine and unusual activity of tyrosinase. This difference from normal tubers is correlated with more rapid reddening and subsequent greater darkening of frozen, ground tissue that is exposed to the air while thawing. The present paper covers an investigation of some of the enzymic factors that may contribute to this abnormality.

### Experimentation

Tuber groups were selected that were uniform in appearance but different in respect to discoloration after cooking, as shown by testing longitudinal sections. These were washed without soaking, rinsed with distilled water, and dried. They were then frozen with solid carbon dioxide, ground in a meat cutter, and allowed to thaw under nitrogen. The latter precaution prevented melanin formation and was taken because of RAPER and WORMALL's (10) finding that formation of melanin at pH 6 sometimes results in the precipitation of a melanin-enzyme complex. When completely thawed, the tissue was pressed and the sap was stored under nitrogen at 0° until used. During this period starch settled out. Unless otherwise stated, borate, phosphate, and phthalate buffers were prepared as directed by CLARK (2), with the exception that sodium salts were substituted for those of potassium in all cases.

### DETERMINATION OF TYROSINASE ACTIVITY

A modification of the method of RAPER and WORMALL (10) was used. Ten-ml. portions of press sap were placed in 500-ml. round bottom flasks containing 100 ml. of a buffered 0.05 per cent. tyrosine solution, 10 ml. of toluene and a few drops of capryl alcohol. The flasks were placed in series for aeration in a water bath maintained at 20° C. A strong current of air, saturated with water, toluene, and capryl alcohol at 20° C. was drawn through the system. At the beginning and at intervals, 5-ml. portions of the test fluid were removed, acidified with 1 ml. of 5 per cent. acetic acid, placed in a boiling water bath for 15 minutes, plugged with cotton, and

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allowed to stand twenty-four hours. They were then filtered, and the precipitate of melanin and protein washed with hot, dilute acetic acid. Thirteen ml. of a saturated sodium carbonate solution were added and the samples allowed to stand one hour. Five ml. of phenol reagent were then added, the sample was diluted to 50 ml., and tyrosine determined as directed by FOLIN and MARENZI (3) for tryptophane. The rate of loss of tyrosine was taken as a measure of tyrosinase activity.

In determining the influence of boiled juice a portion of the press sap was placed on a boiling water bath, stirred until flocculation of protein was complete, filtered, and cooled. When testing the ash, a portion of the sap was evaporated to dryness on a steam bath under a current of air, then ashed carefully. The ash was dissolved in dilute hydrochloric acid, adjusted to the desired pH with sodium hydroxide, and made up to the original volume. An amount of either boiled juice or dissolved ash equivalent to the amount of crude enzyme preparation (10 ml. of press sap) was then added to the reaction flask and an equivalent amount of distilled water to the controls.

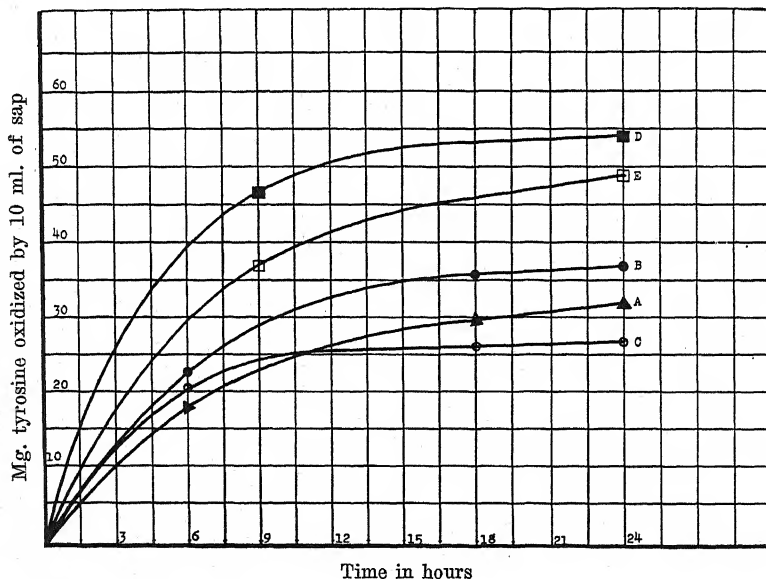


FIG. 1. Comparison of tyrosinase activity in different buffers and at different pH values.

- A—unbuffered.
- B—0.05 M phosphate at 6.0 pH.
- C—0.05 M borate at 6.0 pH.
- D—0.05 M phosphate at 8.0 pH.
- E—0.05 M borate at 8.0 pH.

## EFFECT OF DIFFERENT BUFFERS

Early in this investigation it was found that the buffer used had a marked influence on the activity of the enzyme. Figure 1<sup>2</sup> illustrates the relative activity of tyrosinase preparation in the presence of 0.05 M phosphate and 0.05 M borate at pH values of 6.0 and 8.0. The pH of the unbuffered control (5.97) did not change during the course of the reaction. At pH 6.0 phosphate accelerated the reaction continuously while borate apparently produced an initial accelerating action, which declined sharply after a lapse of several hours. In view of the results at pH 6.0 it seems reasonable to suppose that the difference in activity at pH 8.0 may be attributed both to acceleration by phosphate and to retardation by borate.

An inhibiting action was exerted by phthalate at pH 6.0 throughout the course of the reaction. Acetate and citrate buffers (0.05 M) at pH 6.0 had no effect during the first several hours, but in later stages the reaction ceased. It is believed that this was caused by removal of the enzyme by combination with the melanin, which precipitates at that pH. A mannitol-boric acid buffer prepared according to BRITTON (1) had no effect at pH 7.0.

The concentration of the buffer influenced the rate of enzyme action. Tyrosinase preparations were slightly less active in 0.1 M than in 0.05 M phosphate but a similar increase in borate concentration had a marked inhibitory effect. In some cases enzymatic action was arrested completely by 0.5 M borate. The color changes accompanying the oxidation of tyrosine are much more rapid in phosphate than in borate buffers. In the latter the red stage is prolonged. Unbuffered solutions adjusted to the same pH exhibit intermediate changes.

## TYROSINASE ACTIVITY IN NORMAL AND DISCOLORING TUBERS

Some doubt was cast upon the validity of our earlier determinations of tyrosinase activity (13) by the discovery that the borate buffers used were ineffective in preventing changes in pH. Addition of potato sap to borate buffer of pH 8.0, in the proportions used earlier, resulted in a final pH of 7.0 or lower. The addition of sap of abnormal tubers caused a smaller change than did that of normal tubers, but this difference was seldom over 0.2 pH.

The relative rates of oxidation of tyrosine in phosphate solutions of pH 6.95 and 7.8 were determined. This increase in pH of almost one unit produced an activity in sap from normal tubers equivalent to that in juice of potatoes which discolor. The effect of pH in borate solutions was not tested but comparison of the rates obtained in different experiments gave no indication of greater sensitivity to pH changes.

For more conclusive evidence on the relative tyrosinase activity in nor-

<sup>2</sup> The authors are indebted to JERRY W. MAREK for preparation of the graphs.



mal and discoloring tubers, determinations in which the pH was carefully controlled were made on several lots of potatoes. The pH was adjusted by addition of sodium hydroxide and was determined in each case at the beginning and at the end of an experiment by means of a glass electrode. The results of experiments using both phosphate and borate solutions at pH 7 showed markedly greater oxidation of tyrosine by the sap of abnormal tubers. Similar results were obtained with phthalate, acetate (pH 6.0), mannitol-boric acid (pH 7.0), phosphate (pH 8.0), and unbuffered solutions. In every case the tyrosinase in press sap of discoloring tubers was more active than that of normal ones, regardless of the buffer used or of the pH at which the experiment was conducted.

These results show rather conclusively that potatoes which discolor when cooked possess an unusually high tyrosinase activity. The fact that a difference in rate of oxidation of tyrosine is always obtained, regardless of the buffer or of the pH used in testing, is further evidence that we are measuring an actual inequality either of the states of activation or of the quantities of enzyme in the two kinds of tubers. It is possible that any one buffer might react with some component of one type of potato sap, causing the formation of a complex that affects the enzyme but is non-existent in the tuber itself. If such were the case, it seems unlikely that the same effect would be produced by all the buffers used at the various pH values.

#### COMPARATIVE EFFECT OF BUFFERS ON THE TYROSINASE OF NORMAL AND DISCOLORING TUBERS

It was observed that the greatest differences in tyrosinase activity between normal and discoloring tubers were evident in the unbuffered solutions near pH 6.0 (the pH of the sap) and in certain concentrated buffers. The addition of phosphates, borates, or phthalates of 0.05 M concentration tended to decrease the divergence. When the phosphate concentration was increased to 0.1 M, the difference was further lessened. A similar increase in borate concentration gave the opposite effect. The borate exerted a greater inhibiting action on the tyrosinase in the sap of normal tubers than on that in discoloring ones, resulting in an accentuation of the difference between the two. A still further increase in the concentration of the borate buffer resulted in a complete inactivation of the enzyme in the juice of normal tubers, while that in the sap from discoloring tubers remained moderately active. In one experiment the latter oxidized 18.4 mg. of tyrosine in 24 hours in 0.5 M borate as compared to 33.4 mg. when in 0.1 M borate. This effect may be either partly or entirely due to a difference in enzyme quantity or state of activation, to factors in the sap that modify the action of the buffer on the enzyme, or to other factors whose effect on the enzyme is modified by the buffer. Thus experiments with purified enzyme preparations might give entirely different results.

## EFFECT OF TUBER FACTORS ON TYROSINASE ACTIVITY

HAEHN (5) noticed that boiled potato juice had an activating effect on tyrosinase. He attributed this response to the presence of certain salts and seems to have neglected the effect of additions of the ash upon pH. RAPER and WORMALL (10) also presented evidence for the presence of an activator in boiled sap, but found that it was not present in the ash. In view of these results, we believed it possible that the presence of some such activator in the one, or perhaps of an inhibitor in the other, might account for the difference between discoloring and normal potatoes.

An activator of tyrosinase was found to be present in boiled juice of abnormal tubers. The addition of such juice to a tyrosine-tyrosinase system caused a marked acceleration in the rate of enzyme action, while the boiled juice of normal potatoes had little or no activating effect. This effect was greatest in unbuffered solutions, slightly less marked in borate, less apparent in phthalate and mannitol-boric acid buffers, and non-existent in phosphate.

The activating effect of boiled juice is illustrated in figures 2 and 3 which summarize the results of several experiments. Maximum acceleration due

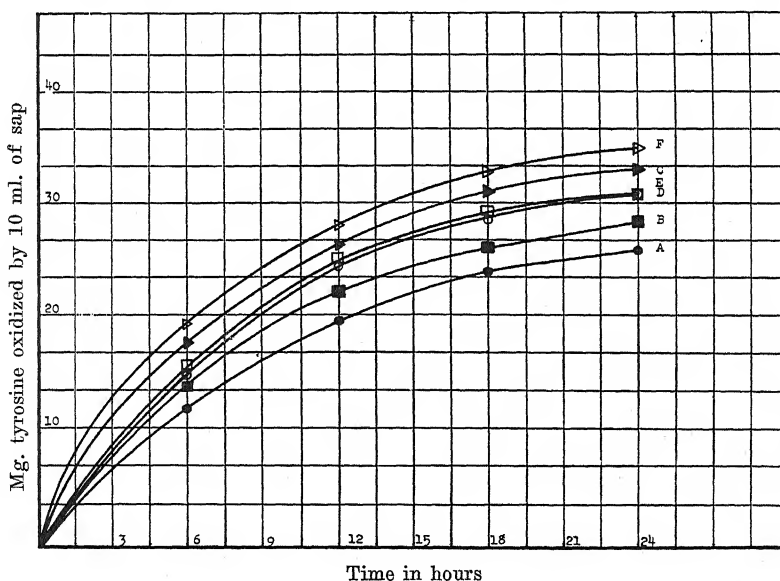


FIG. 2. Effects of boiled sap on tyrosinase activity in 0.05 M borate buffer at pH 8.0.

- A—Normal sap.
- B—Normal sap + boiled normal sap.
- C—Normal sap + boiled discoloring sap.
- D—Discoloring sap.
- E—Discoloring sap + boiled normal sap.
- F—Discoloring sap + boiled discoloring sap.

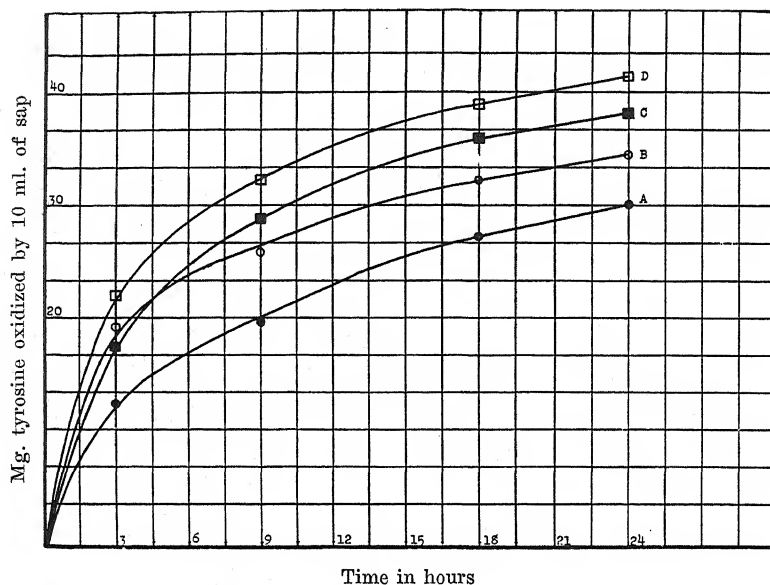


FIG. 3. Effect of boiled sap on tyrosinase in unbuffered solutions at pH 6.0.

- A—Normal sap.
- B—Discoloring sap.
- C—Normal sap + boiled discoloring sap.
- D—Discoloring sap + boiled discoloring sap.

to boiled juice was obtained when the press sap of normal potatoes was used as the source of the enzyme. It was noted that no activator could be detected in potatoes that cooked white, while those that cooked slightly grey often contained the activator in limited amounts. Some of these samples exerted a slight inhibitory action, but the effect was never of such magnitude as to be of significance.

In considering only the results of figure 2, one might conclude that this activator is the only factor responsible for the difference in the tyrosinase activity of the two types of tubers. That this probably is not the case is indicated by the fact that the activator has no effect in phosphate-buffered systems, yet the sap of abnormal tubers is always higher in tyrosinase activity than that of normal ones when tested in such buffers. There was evidence that borates also affect the activator. A greater acceleration due to the addition of boiled juice was apparent in 0.1 M than in 0.05 M borate. In 0.5 M borate the activating effect is yet more pronounced. An enzyme preparation from normal tubers that showed no tyrosinase activity in 0.5 M borate was moderately active if boiled juice from discoloring tubers was added.

The effect of the activator is less apparent at higher pH values than at lower ones. Its nature is as yet unknown. It is rather unstable, as its ability to affect the enzyme lessens upon standing at temperatures as low as 5°. It is partially destroyed by prolonged boiling. When boiled juice containing the activator was dialyzed against distilled water at 5° C. or lower, its ability to activate was lost more rapidly than when stored at the same temperature. The activator is seemingly organic in nature, for it could not be detected in the ash. It is possible, however, that an active inorganic constituent would be altered during the ashing process and thus become inactive. The fact that the compound is unstable also is an indication that it is not an inorganic salt.

The possibility that the accelerating effect of boiled juice might be caused by the slightly increased substrate concentration was considered, for the boiled juice added contained some tyrosine. In some experiments, tyrosine was added to the controls so that their final substrate concentration was equal to, or in excess of, that in the test solution. The results were essentially the same as those previously obtained. It could also be possible that the apparent acceleration was due to oxidation of compounds in the juice that are more rapidly oxidized than tyrosine itself. In practically all cases, however, the flasks to which boiled juice was added contained less tyrosine at the end of the run than did the controls. This was true even though the controls contained less tyrosine at the beginning than did the test solutions. The effect noticed must then be a true acceleration of enzyme action.

#### EFFECT OF SPECIFIC SUBSTANCES ON TYROSINASE ACTIVITY

Our preliminary evidence (13) that potassium may act as an inhibitor of tyrosinase has not been supported by further tests. Addition of amounts of potassium chloride sufficient to equalize the potassium content of the added saps had no effect on the tyrosinase activity.

We have included tests of iron because of the results of TINCKLER (12) and of MADER and MADER (6) indicating that this metal plays a rôle in discoloration after cooking. The addition to digests of either ferrous or ferric chloride in 0.01 per cent. concentration resulted in slight acceleration of tyrosinase activity in 0.1 M phosphate buffer at pH 7.0. As the activator in boiled juice has no effect in phosphate solutions, and since both forms of iron are active but the activator does not appear in the ash, iron is probably not the activator described above.

The addition of a small amount of catechol to a digest in borate buffer resulted in initial rapid melanin formation, as indicated by darkening, but the ultimate disappearance of tyrosine was not altered. It thus appears probable that the acceleration reported by PUGH (9) for both catechol and dihydroxyphenylalanine was temporary and followed by the action of an

inhibitor, as found by GRAUBARD and NELSON (4). As abnormal tubers contain high proportions of free amino acids it seemed possible that the observation of NOBUTANI (8) that these accelerate the oxidation of tyrosine might be significant in blackening after cooking. The addition of glycine in the proportion of one part to five of tyrosine in a borate-buffered digest practically inhibited the oxidation during the first several hours. At the end of 24 hours however the loss of tyrosine was the same as in the controls.

### Summary

1. The tyrosinase activity of potato sap is affected by the particular buffer used in its determination. Phosphate accelerates the oxidation of tyrosine, while borates and phthalates inhibit the reaction. The inhibition is proportional to the concentration of the buffer.

2. The fact that the tyrosinase activity of tubers which discolor is higher than that of normal tubers was further substantiated. The difference in activity was apparent in several different buffers, in unbuffered solutions, and also at different pH values. Greater differentiation was apparent in either borate or unbuffered solutions than in phosphate buffers.

3. An activator of tyrosinase was found in the boiled sap of abnormal potatoes. It is not present in the ash and is lost in prolonged boiling or upon standing, and in dialysis. The activating effect was apparent in unbuffered solutions and in those buffered with borate, but non-existent in phosphate buffers. Since differentiation of the two types of potatoes is apparent even in phosphate buffers, the activator in boiled juice is not the only factor contributing to high tyrosinase activity in abnormal potatoes.

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# CARBOHYDRATES OF WHEAT LEAVES<sup>1</sup>

G. KROTKOV

## Introduction

Previous work on respiration and carbohydrate metabolism of wheat leaves on prolonged starvation (3) revealed the presence in these leaves of two different respiratory substrates, which were provisionally called the primary and the secondary substrates. The primary substrate has been found to be responsible for the production of CO<sub>2</sub> by leaves early in starvation, while its contributions in later stages were little or nothing. On the other hand the secondary substrate, which might or might not be used on the first day, was becoming with the progress of starvation of greater and greater importance, until in later stages either (as in some cases) all the CO<sub>2</sub> emitted by leaves was derived from it, or (as in others) the major portion.

It was shown in the same paper that the primary substrate was represented by sugars extracted from leaves by ethyl alcohol. While nothing definite was said on the nature of the secondary substrate it was suggested that this probably consisted of a variety of substances, some of which are of importance in protoplasmic organization. DELEANO (1) and YEMM (5) reported that proteins serve as the respiratory substrate in later stages of starvation in the leaves of grape and of barley. But before attention is definitely focused on proteins as the source of secondary substrate in wheat leaves, it ought to be determined whether or not some other carbohydrates, which might serve in this capacity, are present in leaves in sufficiently large amounts. NEWTON and BROWN (4), for example, reported that wheat leaves in later stages of maturity contain considerable amounts of pentosans. Such carbohydrates, if present, must remain in the leaf residue after alcoholic extraction, and they might exist there in the two following forms: (1) as hemicelluloses, (hydrolyzable by 1 per cent. H<sub>2</sub>SO<sub>4</sub>); (2) as part of a carbohydrate-protein complex, from which carbohydrates could be liberated on digestion of its protein fraction by trypsin. To test these two possibilities, the experiments reported below were performed.

## Materials, methods, and results

### EXPERIMENT 1

Seed of commercial Marquis wheat was sown in soil in a greenhouse on December 16, 1934, and eleven days later a sample of the plants was taken (sample 1). This consisted of plants cut after a whole day of illumination

<sup>1</sup> Part of the expenses of the experiments herein reported was defrayed by a grant from the Science Research Committee of Queen's University.



(3.4 hours of sunshine). A second sample (sample 2) was taken 18 hours later of plants which were kept in darkness after the taking of the first sample.

Following alcoholic extraction under a reflux condenser of minced plants, which consisted mainly of leaves, residues of both samples were digested either with unboiled or with boiled trypsin using 0.2 M  $\text{Na}_2\text{HPO}_4$  as buffer. After digestion for 6 days at  $37.5^\circ \text{C}$ . filtrates from such digests were cleared in the usual way with basic lead acetate, and analyzed for their reducing power before and after inversion with  $\text{HCl}$  using the HAGEDORN-JENSEN method (2). Table I shows the results of analyses expressed as mg. of glucose liberated per 100 gm. of initial leaf fresh weight.

TABLE I

ANALYSES OF MARQUIS WHEAT LEAVES SHOWING MILLIGRAMS OF GLUCOSE LIBERATED PER 100 GRAMS OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	$\frac{\text{INVERTSUGARS}}{\text{REDUCINGSUGARS}}$
	mg.	mg.	mg.	
Sample 1				
Unboiled trypsin .....	0.105	0.066	0.039	0.59
Boiled trypsin .....	0.021	0.009	0.012	1.33
Difference .....	0.084	0.057	0.027	0.47
Sample 2				
Unboiled trypsin .....	0.143	0.078	0.065	0.83
Boiled trypsin .....	0.054	0.016	0.038	2.37
Difference .....	0.089	0.063	0.026	0.41

## EXPERIMENT 2

Seed of a pure line of variety "Little Club" was sown in soil in a greenhouse during the spring of 1935. Leaves for all samples were always cut after a whole day of illumination, and the age of the plants for the samples 1, 2, and 3 was 13, 9, and 10 days respectively.

Following extraction with alcohol the leaf residues of all the three samples were digested with trypsin, and the digests so obtained were analyzed for their reducing power in the same way as has been described for experiment 1. Table II presents the results of analyses, expressed as percentage of initial leaf fresh weight.

Each sample of leaf residue following tryptic digestion was digested with 250 ml. of 1 per cent.  $\text{H}_2\text{SO}_4$  for three hours in a water bath under a reflux condenser. The filtrates, obtained after such digestion, were neutralized with  $\text{NaOH}$ , cleared in the usual manner with basic lead acetate, and finally analyzed for their reducing power. Table III shows the results of analyses expressed as percentage of initial leaf fresh weight.

TABLE II

ANALYSES OF LITTLE CLUB WHEAT LEAVES SHOWING MILLIGRAMS OF GLUCOSE LIBERATED PER 100 GRAMS OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS REDUCING SUGARS
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	
Sample 1				
Unboiled trypsin .....	0.482	0.316	0.166	0.53
Boiled trypsin .....	0.189	0.052	0.137	2.63
Difference .....	0.293	0.264	0.029	0.11
Sample 2				
Unboiled trypsin .....	0.560	0.315	0.245	0.78
Boiled trypsin .....	0.286	0.080	0.206	2.57
Difference .....	0.274	0.235	0.039	0.17
Sample 3				
Unboiled trypsin .....	0.530	0.302	0.228	0.75
Boiled trypsin .....	0.271	0.095	0.176	1.85
Difference .....	0.259	0.207	0.052	0.25

Examination of tables I and II reveals that the tryptic leaf digests always contained some reducing substances, which were not removed from solutions after their clearing with basic lead acetate. Such substances are usually called sugars.<sup>2</sup>

Three explanations suggest themselves as to the source of reducing substances found in the digests with boiled trypsin: (1) They are some impuri-

TABLE III

COMPARISON OF REDUCING SUBSTANCES PRESENT IN TRYPTIC LEAF DIGESTS TREATED WITH ONE PER CENT.  $H_2SO_4$ . FIGURES GIVEN IN PERCENTAGE OF FRESH WEIGHT

	SAMPLE 1	SAMPLE 2	SAMPLE 3
	%	%	%
First half of sample after digestion with unboiled trypsin .....	0.482	0.560	0.530
Second half of sample after digestion with boiled trypsin .....	0.189	0.286	0.271
Difference (sugars liberated by trypsin) ..	0.293	0.274	0.259
First half of sample digested with one per cent. $H_2SO_4$ .....	0.029	0.057	0.046
Second half of sample digested with one per cent. $H_2SO_4$ .....	0.049	0.095	0.055
Difference .....	0.020	0.038	0.009
Percentage of sugars liberated by trypsin which is also liberated by one per cent. $H_2SO_4$ .....	6.8	13.9	3.5

<sup>2</sup> Work in progress on the isolation and identification of carbohydrates from tryptic digests of leaves has shown that all the digests tested so far gave a very strong positive Molisch test.

ties from the trypsin solution; (2) they are the alcohol-soluble sugars not removed from leaves during alcoholic extraction; (3) they are the products of mild hydrolysis under the action of  $\text{Na}_2\text{HPO}_4$  on some substances of the leaf residue.

In deciding which of the three suggestions is the most probable it is necessary to bear in mind the following facts: (1) ten ml. of the trypsin solution used for each digestion contained about 150 mg. of trypsin. It does not appear probable that this amount of trypsin, after clearing with lead, might contain the amounts of reducing impurities varying from 30 mg. to 61 mg. (calculated as glucose) as has been found by analysis. Moreover, if it does, then the absolute amounts of such impurities must be the same in all the digests, while they actually varied from one case to another. (2) Examination of the  $\frac{\text{invert sugars}}{\text{reducing sugars}}$  ratios of the various digests reveals that this ratio is not constant. In digests with boiled trypsin it is always above unity. Again it is not very probable that reducing impurities from a trypsin solution should be characterized by such a ratio. (3) If the reducing substances present in digests with boiled trypsin are the alcohol-soluble sugars which escaped extraction, then their amounts are excessively high as becomes evident on their comparison with the values for sugars actually removed from leaves in alcoholic extraction. The amounts of total sugars extracted by alcohol in experiment 2 from the samples 1, 2, and 3 were 0.452, 0.361 and 0.716 per cent. respectively. It appears very doubtful that such a large proportion of alcohol-soluble sugars might escape extraction, since in no case was extraction carried out for less than 23 hours and since previous work (3) indicated that this time is ample to extract from wheat leaves the bulk of sugars.

Bearing the above-mentioned facts in mind, it appears more probable that reducing substances present in the digests with boiled trypsin are represented mainly by products of mild hydrolysis of some substances in leaf residue, while reducing impurities introduced in trypsin solution and alcohol-soluble sugars which escaped previous extraction might be found only in negligible amounts.

In experiment 1 the amounts of leaf residue used for the digestion with boiled and unboiled trypsin were not equal. On the assumption that reducing substances of the digests with boiled trypsin consist entirely of products of mild hydrolysis of some carbohydrate-yielding substances, the results of analysis have to be recalculated. Such corrected results are shown in table IV, expressed as percentage of fresh weight.

If we subtract the amounts of reducing substances in digests with boiled trypsin from those found in corresponding digests with unboiled trypsin, we obtain the amounts of sugars, which were liberated from leaves on diges-

TABLE IV

RECALCULATION OF ANALYSES OF LEAF RESIDUES WITH BOILED AND UNBOILED TRYPSIN.  
FIGURES GIVEN AS PERCENTAGE OF FRESH WEIGHT

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS REDUCING SUGARS
	%	%	%	
Sample 1				
Unboiled trypsin .....	0.105	0.066	0.039	0.59
Boiled trypsin .....	0.038	0.015	0.023	1.53
Difference .....	0.067	0.051	0.016	0.31
Sample 2				
Unboiled trypsin .....	0.143	0.078	0.065	0.83
Boiled trypsin .....	0.056	0.016	0.040	2.50
Difference .....	0.087	0.062	0.025	0.40

tion with trypsin solution. These sugars might be liberated either from a carbohydrate-protein complex on digestion of its protein part with trypsin, or from some complex carbohydrates of leaves on their digestion with various polyases present as impurities in the trypsin solution. To explore these two possibilities the following experiment was carried out.

### EXPERIMENT 3

On July 18, 1938, Marquis wheat was sown in soil in a greenhouse and nine days later a large number of plants was taken and extracted for 24 hours with 95 per cent. ethyl alcohol containing  $\text{NH}_3$ . Following extraction, the alcohol was filtered off, the leaf residue was dried and divided into two samples which were digested separately in a water bath for 3 hours with 1 per cent.  $\text{H}_2\text{SO}_4$ . Following such digestion, the leaf residue of each sample was filtered off, dried and divided into two equal parts. One part was digested with the unboiled and the other with the boiled trypsin. All the filtrates obtained from these digestions were cleared and analyzed for their reducing power.

The procedure used in this experiment was exactly the same as that used in the earlier experiments with only one exception: neutral, instead of basic lead acetate, was used for clearing. Table V shows the results of analyses expressed as percentage of initial leaf fresh weight.

Examination of table V reveals two facts: (1)  $\text{H}_2\text{SO}_4$  digestion liberated considerably more reducing substances than had been found in the earlier experiments. This increase is probably caused partly by hydrolysis of some substances which in the earlier tests were hydrolyzed by the  $\text{Na}_2\text{HPO}_4$  buffer, and partly to different environmental conditions existing during the growth of plants in this experiment. (2) Digests with the unboiled trypsin still showed an excess of reducing substances over corresponding digests with the

TABLE V

REDUCING POWER OF LEAF RESIDUES DIGESTED WITH 1 PER CENT.  $\text{H}_2\text{SO}_4$  FOLLOWED BY BOILED AND UNBOILED TRYPSIN

TESTS	TOTAL SUGARS	REDUCING SUGARS	INVERT SUGARS	INVERT SUGARS REDUCING SUGARS
	%	%	%	
Sample 1				
$\text{H}_2\text{SO}_4$ , 1 per cent. ....	0.566			
Unboiled trypsin .....	0.276	0.165	0.111	0.672
Boiled trypsin .....	0.143	0.076	0.067	0.881
Difference .....	0.133	0.089	0.044	0.494
Sample 2				
$\text{H}_2\text{SO}_4$ , 1 per cent. ....	0.518			
Unboiled trypsin .....	0.264	0.161	0.103	0.639
Boiled trypsin .....	0.136	0.080	0.057	0.712
Difference .....	0.128	0.081	0.046	0.567

boiled trypsin. Thus there are some substances in the leaf residue, which are broken down not by 1 per cent.  $\text{H}_2\text{SO}_4$ , but by trypsin, and from which are liberated reducing substances, called sugars according to our present-day terminology. This is equivalent to the statement that in the leaves there is a carbohydrate-protein complex, from which sugars can be liberated only by tryptic digestion of its protein component.

This conclusion does not exclude a possibility, that in experiments 1 and 2 some of the sugars found in the digests with the unboiled trypsin, were liberated there not by trypsin but by polyase impurities of the trypsin solution. Consequently in these experiments values for the sugars liberated from the carbohydrate-protein complex must be taken as the maximal values. In reality they might be lower.

It was shown previously (3) that starvation sequence in wheat leaves was brought to an end after a definite amount of the secondary substrate has been degraded. For 100 gm. of leaf fresh weight this amount is represented by  $2748 \pm 235$  mg.<sup>3</sup> in  $\text{CO}_2$  equivalents, or  $1874 \pm 160$  mg. as glucose. On the other hand, the maximal amounts of the sugars which could be liberated from the leaf residues by trypsin,  $\text{Na}_2\text{HPO}_4$  and 1 per cent.  $\text{H}_2\text{SO}_4$  are represented in experiment 2 for the samples 1, 2, and 3 by 511, 617, and 576 mg. of glucose respectively. For both samples of experiment 1 such amounts probably would be even lower, considering that the values for the sugars liberated by 1 per cent.  $\text{H}_2\text{SO}_4$  are small. We are forced, therefore, to conclude that leaf residues, following alcoholic extraction, do not contain easily hydrolyzable substances from which sugars could be liberated in amounts large enough to account for more than one-third of the secondary substrate. We must look, consequently, for some other substances besides carbohydrates

<sup>3</sup> Standard deviation has been computed with (N-1) degrees of freedom.

in our attempt to determine the nature of the materials forming the bulk of the secondary substrate.

### Summary

1. Following alcoholic extraction of wheat leaves in the stage of early maturity the residue was found to liberate sugars after being hydrolyzed with  $\text{Na}_2\text{HPO}_4$ , digested with trypsin and, finally, after hydrolysis with 1 per cent.  $\text{H}_2\text{SO}_4$ .

2. While alcohol-soluble sugars, as well as those which are the products of mild hydrolysis with  $\text{Na}_2\text{HPO}_4$  are characterized by the invert sugars reducing sugars ratio above unity, sugars liberated on tryptic digestion have a preponderance of reducing over invert sugars.

3. It has been shown that the union between the component parts of the carbohydrate-protein complex is of such a nature, that it is not broken down on digestion with 1 per cent.  $\text{H}_2\text{SO}_4$ , and that sugars can be liberated from such a complex only after tryptic digestion of its protein fraction.

4. Present work failed to reveal any substances in leaf residue from which sugars can be easily liberated in sufficient amounts to account for the entire secondary respiratory substrate.

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# STIMULATION OF GERMINATION OF DORMANT LETTUCE SEED BY SULPHUR COMPOUNDS

ROSS C. THOMPSON AND WILLIAM F. KOSAR

(WITH ONE FIGURE)

Numerous agents have been reported as effective in promoting germination of dormant lettuce seed. BORTHWICK and ROBBINS (1) found that the percentage of lettuce seed germinating at temperatures above 25° C. is increased by high oxygen pressure. THORNTON (17) reported that good germination was obtained at temperatures as high as 35° C. if the surrounding atmosphere contained from 40 to 80 per cent. of carbon dioxide. SHUCK (14, 15), FLINT (7), and FLINT and McALISTER (8) have reported on the influence of light on the germination of dormant lettuce seed.

DENNY (2, 3) and MILLER (13) have studied the influence of certain sulphur compounds on the breaking of dormancy in the potato tuber. That certain sulphur compounds are also effective in breaking dormancy in lettuce seed was reported by the writers (16). The present paper is a more complete report of studies made on the influence of chemicals in stimulating germination of lettuce seed under conditions that result in poor germination if the seed is not given some special treatment.

## Material and methods

In making these studies, aqueous solutions of the chemicals were used as media in which the seeds were germinated. The tests were all made in 95-mm. Petri dishes. A piece of filter paper was first placed on the bottom of each dish. Fifty seeds were scattered on the paper and two ml. of the solution to be tested were added by means of a measuring pipette. The dishes were covered and placed immediately in a germinator in darkness at 24° to 26° C. The dishes were removed and the germinated seeds counted after 72 hours. Each test was run in quadruplicate.

The chemicals that have been tested for their influence on the germination of lettuce seed include acetamide, thioacetamide, allyl urea, allyl thiourea, ammonium sulphate, ammonium thiocyanate, asparagin, calcium thiocyanate, potassium thiocyanate, potassium cyanide, potassium ferricyanide, potassium ferrocyanide, semi-carbazide hydrochloride, thio-semicarbazide, sodium nitrate, sodium thiocyanate, sulphuric acid, urea, thiourea, ammonium nitrite, hydrazine, and sulphanilic acid.

Only chemically-pure materials were used. Each chemical was first tested in various strengths of aqueous solution to determine the concentration giving the highest percentage of germination. A few of the materials were injurious at concentrations as low as 0.1 per cent. Nearly all were injurious in 1.0 per cent. solutions.



Six of the chemicals tested gave very marked increases in germination over the untreated checks. The chemicals having a marked stimulating influence and their optimum concentration are as follows: thioacetamide, 0.4 per cent., and thiourea, allyl thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate, 0.5 per cent. Sodium thiocyanate and calcium thiocyanate gave some significant increase in germination on some lots of seed but neither of these had the stimulating influence exhibited by the six above-mentioned chemicals. All of the other chemicals retarded germination in most cases except in very dilute concentrations.

### Experimentation

#### RELATIVE EFFECTIVENESS OF CHEMICALS IN STIMULATING GERMINATION

After the optimum concentration of the chemicals had been determined, a series of tests, hereafter known as series I, was made, using the six most effective chemicals—allyl thiourea, thioacetamide, thiourea, thio-semicarbazide, ammonium thiocyanate, and potassium thiocyanate. The results from this series of tests are given in tables I and II.

TABLE I

GERMINATION OF CHEMICALLY-TREATED SEED OF FOUR LETTUCE SEED STOCKS  
SERIES I

TREATMENT	GERMINATION IN PERCENTAGE OF 200 SEEDS IN 4 REPLICATIONS				MEAN
	SEED STOCK NUMBER				
	1	2	3	4	
	%	%	%	%	%
Check* .....	79.0	4.0	0.0	100.0	45.75
Thiourea .....	99.5	78.0	18.0	100.0	73.86
Thio-semicarbazide .....	93.0	55.0	30.0	98.0	69.00
Thioacetamide .....	87.0	70.5	0.0	96.0	63.38
Allyl thiourea .....	67.5	63.0	0.5	76.0	51.88
Ammonium thiocyanate ...	51.0	47.5	0.5	95.5	48.63
Potassium thiocyanate ...	45.5	15.0	0.5	94.0	38.63
Mean .....	74.64	47.71	7.0	94.14	

\* Differences required to show significance are as follows: treatments, 5.58 per cent.; stocks, 4.24 per cent.

Four lots of seed were used. Three of these showed some degree of dormancy when untreated and the fourth was a non-dormant lot giving a check test of 100 per cent. germination on filter paper moistened with water. The three dormant lots were numbered 1, 2, and 3, and the non-dormant stock no. 4. The dormant lots gave check germination tests of 79.0, 4.0, and 0.0 per cent., respectively. The non-dormant lot was included to determine the influence of the chemicals on seed not normally dormant.

TABLE II

ANALYSIS OF VARIANCE OF DATA FROM SERIES I, TESTING THE RELATIVE INFLUENCE OF SIX CHEMICALS IN STIMULATING GERMINATION OF LETTUCE SEED

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Stocks .....	3	9,984.72
Treatments .....	6	603.68
Replications .....	3	50.48
Stocks $\times$ treatments .....	18	275.06
Error .....	81	15.61
Total .....	111	

The different seed stocks varied greatly in their response to treatments, as indicated by the large mean square value for stocks in table II.

The highly significant variance for interaction between stocks and treatments shows definite differences among stocks in response to a given treatment. Variance due to treatment alone is not significantly greater than interaction so it is not possible to generalize with reference to the relative effectiveness of treatment for any specific lot of seed. Variance due to stocks, however, is significantly greater than interaction, showing that these stocks, in general, maintain their relative responsiveness to individual treatments.

#### IMPORTANCE OF SULPHUR IN THE COMPOUND

Of the six chemicals having a marked stimulating influence on germination, two are inorganic—ammonium thiocyanate and potassium thiocyanate—and four, allyl thiourea, thioacetamide, thio-semicarbazide, and thiourea, are organic. The compounds in the former group have the CNS linkage in common and those in the latter group all have the amino  $\text{NH}_2$  linked to the CS. All six have sulphur in common. The importance of sulphur in the compound is indicated by the results obtained from series II of tests in which the thio-compounds were tested with similar compounds in which sulphur is absent. In this series, thioacetamide was compared with acetamide, thiourea with urea, allyl thiourea with allyl urea, and potassium thiocyanate with potassium cyanide. In each case the most effective concentration of the chemical was used. No ammonium cyanide or semi-carbazide was available for testing with ammonium thiocyanate and thio-semicarbazide. The results of this series of tests are presented in tables III and IV. In each of the four comparisons made, the absence of sulphur from the compound not only resulted in no stimulation of germination but in every case retarded germination in concentrations of 0.1 per cent. or higher.

These results (tables III and IV) show clearly the need of sulphur in the compound for its stimulating influence on germination. The presence of sulphur in the compound is not the only condition necessary, as is shown by the failure of ammonium sulphate, calcium sulphate, potassium sulphate,

TABLE III

GERMINATION OF LETTUCE SEED TREATED WITH CHEMICALS WITH AND WITHOUT SULPHUR  
SERIES II

TREATMENTS*	GERMINATION IN PERCENTAGE (200 SEEDS IN 4 REPLICATIONS)			MEAN
	SEED STOCK NUMBER			
	1	2	3	
	%	%	%	%
Thiourea .....	96.0	56.5	8.5	53.67
Urea .....	20.5	0.0	0.0	6.83
Allyl thiourea .....	89.5	39.0	0.5	43.00
Allyl urea .....	8.0	0.0	0.0	2.67
Thio-acetamide .....	96.0	48.0	0.0	48.17
Acetamide .....	18.5	0.0	1.5	8.33
Potassium thiocyanate .....	84.0	5.0	0.0	29.67
Potassium cyanide .....	19.0	1.0	0.5	6.83
Check .....	22.5	1.0	2.0	8.50
Mean .....	51.00	16.78	1.44	

\* Differences required for significance are as follows: treatments, 3.54 per cent.; stocks, 2.32 per cent.

and sulphuric acid in any concentration between 0.1 and 1.0 per cent. to promote germination. The presence of C, N, and S in some specific linkage is apparently necessary.

TABLE IV

ANALYSIS OF VARIANCE OF DATA FROM SERIES II, TESTING THE INFLUENCE  
OF SULPHUR IN THE COMPOUND

SOURCE	DEGREES OF FREEDOM	MEAN SQUARE
Treatments .....	8	1,267.40
Stocks .....	2	5,793.03
Replications .....	3	4.06
Treatments $\times$ stocks .....	16	404.63
Error .....	78	4.71
Total .....	107	

Although the interaction between stocks and treatments is significant with reference to error, the variances due to stocks and treatments are both significantly greater than interaction. Thus, the influence of treatments and the behavior of stocks were generally quite consistent.

### Discussion

Many tests in which chemicals were used show that thiourea is the most generally effective material in promoting germination of dormant lettuce seed. Thiourea in the 0.5 per cent. concentration resulted in some stimulation of germination in every lot of dormant seed on which it has been used.

In some cases it has given nearly 100 per cent. germination on seed that was completely dormant in water under the same conditions of temperature and light.

The other five compounds—allyl thiourea, ammonium thiocyanate, potassium thiocyanate, thio-semicarbazide, and thioacetamide—were more variable in their effect on different lots of dormant seed. On some lots their stimulating effect was very great, while other lots were not stimulated by the chemical.

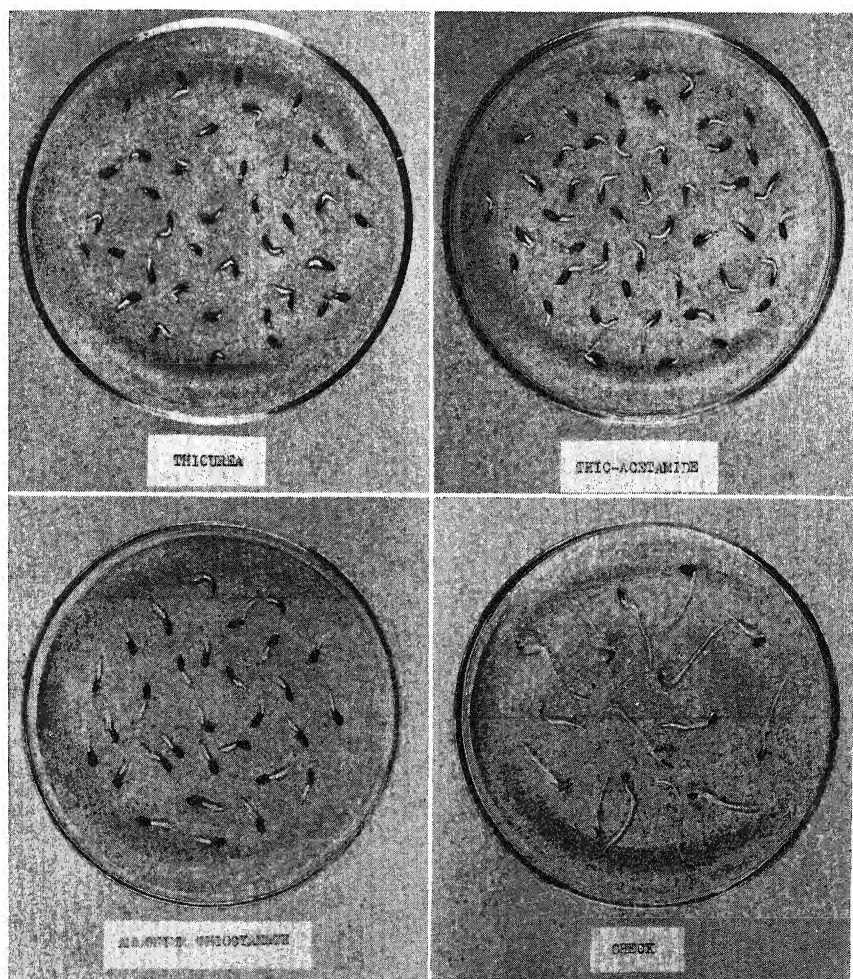


FIG. 1. Sprouted lettuce seed after 72 hours in darkness at 25° C. A, in 0.5 per cent. thiourea; B, in 0.4 per cent. thioacetamide; C, in 0.5 per cent. ammonium thiocyanate; D, in tap water.

In order of general effectiveness in stimulating germination on all of the various seed samples studied, the compounds rank in the following descending order: thiourea, thio-semicarbazide, thioacetamide, allyl thiourea, ammonium thiocyanate, and potassium thiocyanate. The last two named materials have a very marked influence on some lots of seed but on many samples they have little effect. Potassium thiocyanate is the most toxic and causes considerable injury to the radicle in some cases.

All of these compounds except ammonium thiocyanate have a very marked retarding effect on hypocotyl and radicle elongation and on root hair development. With ammonium thiocyanate, radicle and hypocotyl elongation and root hair development are about normal except for a slightly slower rate of growth. The type of growth resulting from the use of thiourea, thioacetamide, thio-semicarbazide, allyl thiourea, and potassium thiocyanate is very similar to that reported by BORTHWICK and ROBBINS (1) as resulting when lettuce seed is germinated under high oxygen pressure. The growth-inhibiting effect of some of the compounds used is shown in figure 1.

With the exception of this semi-carbazide, the inhibitory effect of these chemicals is not permanent. When the seeds were removed from the chemical solutions as soon as they had germinated, and planted in soil, the treated lots emerged from the soil and developed as rapidly as the seed germinated in water, with the exception of those germinated in thio-semicarbazide. Seed treated with this material failed to emerge. The rôle of these sulphur compounds in the germination of lettuce seed is not known, and no attempt has been made in the present studies to determine their function in the process of germination. DRYERRE (6), HANES and BARKER (9, 10), JOHNSON and WORMALL (11), DENNY (4, 5), MILLER (13), and others have studied the effect of certain of the cyanides and cyanates on enzyme activity. Enzyme activity has been found to increase in the presence of KCN and KSCN. The results reported on the effect of these materials on enzyme activity suggest that the sulphur compounds found to have a stimulating influence on the germination of dormant lettuce seed may have some similar function in the process of germination of lettuce seed.

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## SOME FACTORS CONTRIBUTING TO TOMATO PUFFING<sup>1</sup>

J. J. TAUBENHAUS<sup>2</sup> AND G. E. ALTSTATT

Tomato puffing is a common fruit defect causing serious annual losses in Texas. The trouble is known by several names, such as "tomato puffs," "tomato pops," "puffy tomatoes," "puffs," and "pockets." The term tomato puffing, as is now generally used by tomato growers wherever the defect occurs, is preferred.

In 1895 PRICE and NESS (6) of the Texas Agricultural Experiment Station gave the first clear description of tomato puffing of the variety "Terra cotta" in which they seem to have accepted puffing as a varietal characteristic. In 1926 LESLEY and ROSA (5) recognized puffing as a defect of some tomato crosses grown at Riverside, California. WEBER and RAMSEY (15) found the same trouble causing serious damage in Florida-grown tomatoes. TAUBENHAUS (7) in 1929, reported some preliminary observations on puffed tomatoes. Since then, TRAUB *et al.* (13, 14) and YARNELL *et al.* (17 to 25) and CORNS (1) have added much to our knowledge of this trouble.

The most typical symptom of a puffed tomato is its angular appearance, with slightly indented walls which yield readily to the least pressure. In some varieties the flatness of the sides is not so conspicuous, but the light weight and the hollow "feel" of the fruit is a sure indication of puffing. A severely puffed fruit upon being sliced shows a partial to complete abortion of the placental tissues, which accounts for the hollow interior. Sometimes the locular walls are much proliferated and hardened. Tomatoes in which the hollow cavity is negligible are classified as no. 1 fruits and marketable, while fruits with prominent cavities are classed as culls.

To determine the prevalence of tomato puffing in the United States in 1933, a questionnaire was sent to plant pathologists and horticulturists in every state as well as in Alaska, Hawaii, Puerto Rico, and the Virgin Islands. The replies received reported tomato puffing of field-grown tomatoes in Alabama, Arkansas, California, Louisiana, Mississippi, and Texas, but only of greenhouse-grown tomatoes in Illinois and Michigan. During 1924, field losses were estimated at 14 per cent.; in 1925, 15 per cent.; in 1926, 20 per cent.; in 1927, 2 per cent.; in 1928 and 1929, 8 per cent.; in

<sup>1</sup> Published with the approval of the Director as contribution no. 440, Technical Series, of the Texas Agricultural Experiment Station.

<sup>2</sup> Deceased. This paper is one of the last written by the late Dr. J. J. TAUBENHAUS. It represents several years of experimentation and observation. One will note the habit of making large numbers of samples and cultures, so typical of his work. Not a hundred cultures, but two thousand; not a hundred fruits, but many bushels of them were examined. Since the original manuscript left his hands, only the changes suggested by readers of the manuscript have been made by the junior author.



1930, 14 per cent. of the commercial Texas crop. Tomato growers and packers in Texas generally agree that losses from tomato puffing may vary not only from year to year, but even in the same season, depending on weather conditions. After a heavy or prolonged rainy spell greater culling is necessitated in both field and packing sheds, due to unusual increases in puffed tomatoes, while during dry weather, tomato puffing, although present, is generally unimportant.

It is generally believed by growers that puffing becomes noticeable only when the tomato fruits are fully developed and just before they begin to color. In order to determine whether puffing is a defect of the maturing fruit only, a systematic survey (8) was carried out during 1930 at 18 different locations in Texas. Tomato fruits in all stages of development were cut and the degree of puffing recorded. It was found that tomato puffing in its earliest and severest occurrence was just as prevalent in young embryonic fruit, even only 1.0 to 2.5 mm. in diameter, as in fully developed and maturing tomatoes.

As to susceptibility, YARNELL (17, 18, 22) found considerable variation among different tomato varieties; the Marglobe was the most susceptible, while the John Baer was the most resistant. The writers found that the John Baer variety was very susceptible to tomato puffing when grown under irrigation in the Winter Garden section of Texas. Soil moisture conditions seem to affect the susceptibility of a variety because during 1930 the Marglobe, a highly susceptible variety, showed 19 per cent. affected fruit when grown near Troup, 22 per cent. at Weslaco, and 46 per cent. when grown near Bryan, Texas.

Opinion as to the cause of puffing differs greatly. Some growers claim that the trouble is favored by the use of excessive amounts of fertilizer, particularly nitrate of soda, while others believe moisture to be the cause, since the greatest losses occur in seasons of continuous precipitation or after a heavy rain following a prolonged dry spell. CORNS (1) found that in greenhouse studies puffing was associated with poor pollination, but that pollination was not a factor under field conditions. FOSTER and TATMAN (2) after 5 years of study on puffing of tomatoes in the greenhouse, concluded that growth-limiting factors such as soil moisture, soil nutrients, and air temperature or combinations of these, may interfere with the normal development of the fruits.

During 1928 and 1929, about 2000 plate cultures on potato-dextrose agar were made from tissue of lightly to severely puffed tomato fruit, and in not a single instance was an infectious organism recovered. With no indication of a causal organism, one might first suspect that tomato puffing is either of a physiological nature or is induced by some virus. In order to determine whether tomato puffing is of a virus nature (8) Marglobe tomato

seeds were obtained during 1929 from apparently normal fruits. The next spring (1930) they were disinfected for 5 minutes in 1:2000 mercuric chloride, and planted in insect-proof cages at College Station. This test was repeated in 1931. During both years the plants in all the cages were hand pollinated to insure a crop of tomato fruits. About the middle of the growing season the plants in some of the cages were inoculated with freshly extracted, filtered juice obtained from freshly gathered, severely puffed tomatoes. This juice was passed through a Berkfeld filter, and used as inoculum, being either rubbed into the leaves and stems with a sterile brush, or injected with a hypodermic syringe. Check plants were rubbed or injected with distilled sterilized water. A small number of the inoculated and uninoculated plants in the cages showed mosaic. Puffed fruits occurred in all of the cages and on all the tomato plants, whether inoculated or not and whether naturally infected by mosaic or not. This suggests that tomato puffing is not caused by a virus, as otherwise the uninoculated and the non-mosaic-infected plants in the check cages should have produced normal tomatoes, which was not the case.

In 1932 (10) seed of Marglobe tomatoes were obtained from Wethersfield, Connecticut, where puffing is not known to occur. These seeds were surface sterilized for 5 min. in a 1:2000 mercuric chloride solution, planted in insect-proof cages at College Station, and thinned to a stand of nine plants per cage. The blossoms of all the caged plants were hand pollinated. A high percentage of puffed fruit occurred on all of the plants, whether these plants were naturally infected by mosaic or not. These results further suggest that puffing is not caused by any seed-borne virus, but they point to the probability of some environmental factors as the cause.

Seed from the same lot was surface sterilized and planted in steam-sterilized and unsterilized Lufkin fine sandy loam. All plants were dusted with insecticides frequently to keep them free of insects. Puffed fruit developed on all plants, regardless of soil treatment, indicating that puffing is probably not of an insect-borne virus nature.

If tomato puffing is not caused by a pathogenic organism or by a virus, then is it a trouble brought about by certain factors of environment acting on particular hereditary complexes? Several factors have thus far been tested including soil acidity, fertilizers, soil moisture, geographical location, and the effects of other diseases upon puffing.

During 1933 (12), Marglobe tomato plants were grown at College Station, in wooden containers which were filled with different known soil type materials and of known degrees of acidity. Container 1 was filled with Susquehanna fine sandy loam having a pH of 5.5; container 2 was filled with Tabor fine sandy loam with a pH of 6.7; container 3 was filled with Kirvin fine sandy loam with a pH of 7.1; and container 4 was filled with

Houston black clay soil with a pH of 7.7. No significant difference in the percentage of puffed fruits occurred in the different soil types used. In other experiments, earthenware cylinders filled with Lufkin fine sandy loam were adjusted to acidities varying from pH 4.9 to pH 8.5. A total of 4 cylinders were used for each pH adjustment and a single tomato plant was planted in each cylinder. There appeared to be no correlation between the soil acidity and the amount of puffed fruit in each cylinder.

FRIEND (3, 4) found that differential fertilizer treatments had no significant influence on puffing. YARNELL *et al.* (17 to 25), on the other hand, stated: "Differential fertilizer treatments did not affect the amount of puffing in the Lower Rio Grande Valley, although a difference was found at College Station," which might be explained by differences in soil fertility. The same authors also found that several varieties of tomatoes always produced less puff when fertilized with a 6-12-6 fertilizer.

In 1933, super-phosphate, sulphate of potash, and nitrate of soda in definite amounts and in various combinations were added to Lufkin fine sandy loam soil in galvanized cylinders which were set in the ground at College Station, Texas. A single Marglobe tomato plant was planted in each cylinder and regularly watered twice a week from June 1 to November 1, except during rainy weather. Puffed fruits were found on all plants irrespective of the fertilizer added to the soil.

The possible relationship of soil moisture to tomato puffing has been long suspected, as previously indicated by Texas and Florida tomato growers. Although the literature on this relationship is somewhat conflicting, the trend of experiments by various workers indicates a correlation of the amount of puffed fruit with the amount of moisture present in the soil. FRIEND (3, 4) found that the yield was greater and the percentage of puffy fruit lessened from plants receiving greater amounts of water at frequent intervals, whereas YARNELL (17 to 25) found: "In general the amount of puffing is greater where there is more available moisture." WOOD (16) found that tomatoes grown without irrigation showed considerably less puffing than those grown with irrigation. This was especially true with the varieties Pritchard and Marglobe. During 1930, observations were made at Arp, Tyler, and Prairie View of tomatoes grown on dry upland soil, moist lowland soil, and under conditions of overhead irrigation. Lower percentages of severely puffed fruit occurred on plants growing on upland dry tight soil, while high percentages of puffed fruit were found on plants growing in seepy lowlands or on plants grown directly under irrigation. In 1932 and 1933 (10), Marglobe tomatoes were grown at College Station in plats receiving weekly irrigations and in plats without irrigation. The greatest percentage of normal tomatoes was produced on individual plants growing without irrigation. These experiments further indicate the relationship of soil moisture to tomato puffing.

It has been previously mentioned that there are certain sections in the United States where tomato puffing is unknown. Tomato seeds were obtained in 1932 from these sections. For comparison, tomato seed was also obtained from plants grown in Florida and Texas where annual losses from tomato puffing are heavy. The plants from each of these sources of seed were divided into two lots, eight of which were grown under irrigation and eight without irrigation. Irrespective of origin of the seed, puffing occurred in all strains and varieties, particularly when grown under irrigation. These studies suggest that puffing may be caused by various temperature, soil, or moisture conditions or other factors peculiar to some of the southern states. Tomato seed from sections where tomato puffing never occurs did not produce plants entirely free from affected fruit.

It was thought that the presence of other diseases on the tomato plant might be a factor in affecting the amount of puffed fruit. TRAUB (13) observed that mosaic-infected tomato plants produced more puffed fruits than normal plants. In our inoculation experiments tomato puffing appeared equally prevalent irrespective of natural mosaic infection. Tomato fields were examined in 1930, 1931, and 1932 to determine whether there was any correlation between the number of puffed tomato fruits per plant, and the infection of that plant by certain other organisms. Records were made of mature plants affected by mosaic (virus), Fusarium wilt (*F. lycopersici*), Septoria leaf spot (*S. lycopersici*), Alternaria leaf spot (*A. solani*), Cladosporium leaf mold (*C. fulvum*), and bacterial canker (*Aplanobacter michiganense*). Puffing occurred equally as often on plants infected with these diseases as on those which were non-infected. YARNELL *et al.* (25) found no relationship between the amount of puffing and injury to the plants by *Sclerotium rolfsii*. The same writers also observed that there was no association between blossom-end rot, a physiological disease caused by abnormal water relations, and the amount of puffing.

### Summary

Tomato puffing is a fruit defect causing annual losses of 8 to 15 per cent. of the tomato crop in Texas. Affected fruits are light in weight, angular, and flat-sided, and more or less hollow. Puffing begins in the embryonic stage and progresses with the development of the fruit; the symptoms, however, are more prominent in fully developed tomatoes.

The cause of puffing is still unknown. The studies here reported seem to show that it is not caused by a microorganism nor a virus. Tomato puffing does not appear to be seed- or soil-borne, nor is it influenced by other diseases attacking the same plant, nor by soil acidity. The defect is influenced by soil moisture and probably by certain fertilizers, as well as some environmental

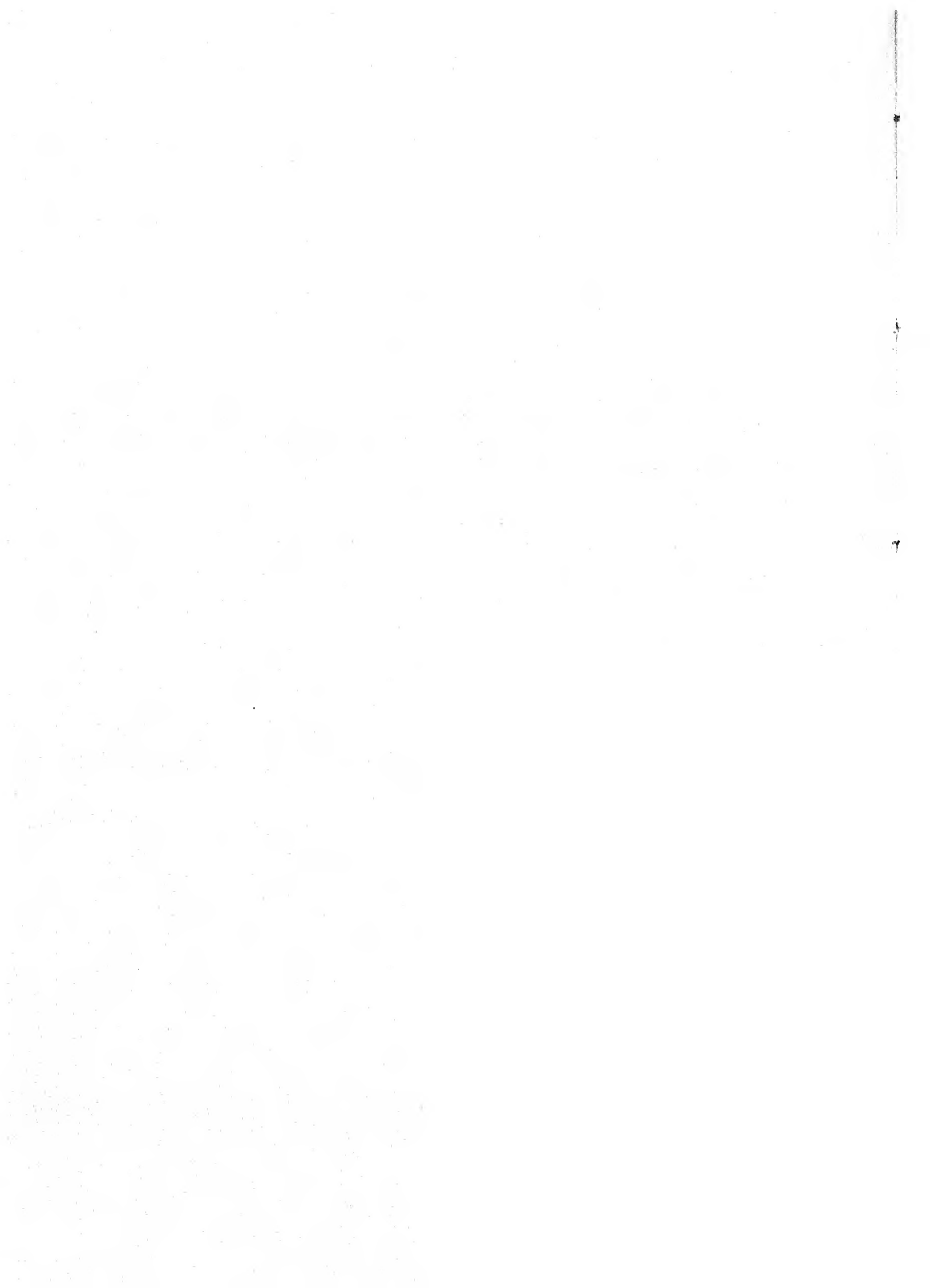
conditions. Irrigated plants produced a higher percentage of puffed fruits than did plants grown without irrigation.

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# ELECTRODIALYSIS OF PEA SEEDS

WENDELL R. MULLISON

(WITH THREE FIGURES)

## Introduction

Electrodialysis differs from ordinary dialysis in that an electric current is passed through the dialyzing cell and the impressed electrical potential hastens the removal of ions from colloidal material. Consequently, electrolytes are removed faster and to a greater degree than by ordinary dialysis.

In the last twenty-five years electrodialysis has been used extensively in the study of the composition and purification of biological materials. PAULI (4) was the first worker to use this technique in the purification of proteins, whereas now it is a routine procedure. It has been applied extensively in the study of soil colloids and in general is the best method for the purification of lyophilic colloids.

The technique has been used recently in other ways. MOORE, REEVES, and HIXON (2) electrodialyzed normal and abnormal apple tissue in an attempt to determine the cause of spotting which occurs in Jonathan apples during cold storage. NELLER (3) also used electrodialysis in a study of apple tissue. More recently COOPER, PADEN, and SMITH (1) used the same method in treating fresh samples of cotton, corn, and soy bean tissue in order to determine the ease of removal of various cations.

The present study was undertaken in order to determine the effect of electrodialysis upon the subsequent development of seedlings.

## Methods and material

The apparatus consisted of a water-tight wooden box so constructed that it could be divided into three compartments upon the insertion of two cellophane partitions. The inside of the box was thoroughly impregnated with paraffin to prevent the withdrawal of any electrolytes from the wood. The cellophane used was Du Pont's non-waterproofed no. 300. The electrodes were graphite plates and the source of current was an 80-volt Edison storage battery. The temperature of the apparatus was kept below 30° C. by means of running cold water through a glass-tube cooling system.

Pea seeds of the Alaska variety were used. They were placed under germinating conditions for twelve hours before being dialyzed. The seeds were then divided into two groups of 75 or 100 each. One group was placed in the middle compartment of the electrodialyzing cell and 100 ml. of distilled water was then added to each compartment. The control group was placed in a beaker containing the same amount of distilled water and left submerged for a period equal to the time of dialysis.



### Experimentation

**PETRI DISH CULTURES.**—Sixteen hundred seeds were used during various repetitions of this work. Lots of one hundred seeds were used. Six seeds of each lot were removed from the dialysis chamber every fifteen minutes up to one hour, and then at intervals of thirty minutes up to three hours. The remaining seeds were discarded, having served the purpose of increasing the amount of current passing through the cell. As each sample and its control were removed they were placed on filter paper, moistened with distilled water, and placed in Petri dishes to germinate. Those seeds which had been dialyzed longer than one hour had a yellowish cast; those dialyzed for three hours had this discoloration to a pronounced degree. The percentage of germination varied from about 100 per cent. in the controls to about 70 per cent. for the seeds dialyzed for one hour; with three hours of continuous dialysis the germination rate was reduced to about 20 per cent.

After germination, the differences were manifested in other ways. Seeds

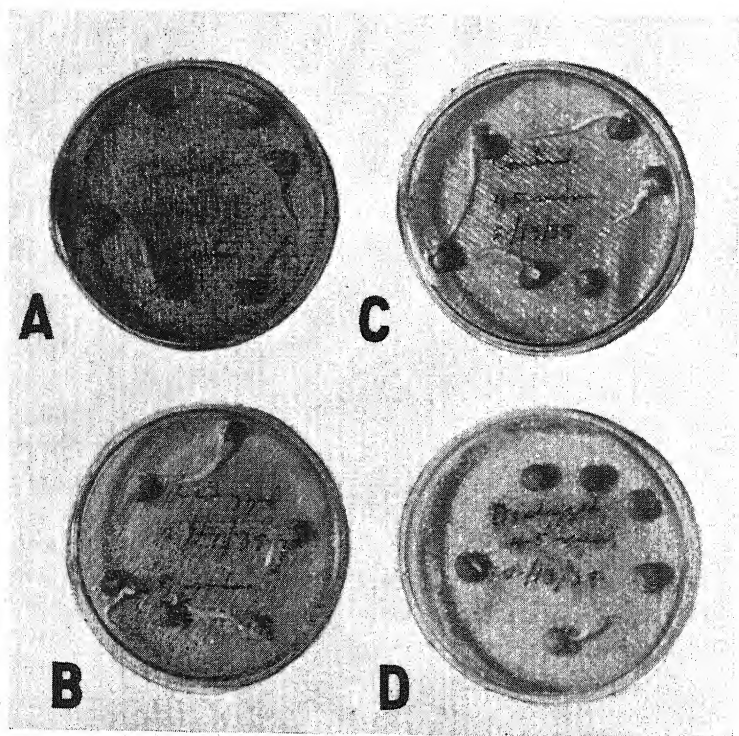


FIG. 1. Growth of pea following electrodialysis of the seeds; age 1 day. A. Control; soaked 15 minutes without dialysis. B. Electrodialyzed 15 minutes. C. Control; soaked 45 minutes without dialysis. D. Electrodialyzed 45 minutes.

dialyzed for periods of more than one hour showed a slower rate of germination, a slower rate of growth that often stopped entirely after a short time, and a marked susceptibility to infection from fungi (see figs. 1 and 2).

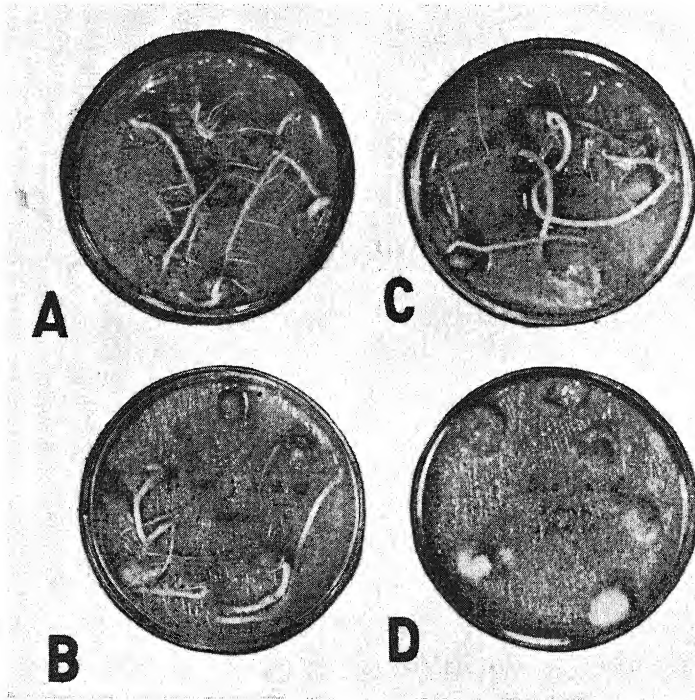


FIG. 2. Growth of pea seedlings from electrodialyzed seeds; age 7 days. A. Control; soaked 1 hour without dialysis. B. Electrodialyzed 1 hour. C. Control; soaked 3 hours without dialysis. D. Electrodialyzed 3 hours.

Indeed, the latter was so marked that after a few days' germination the Petri dishes containing the dialyzed seeds could be identified by the heavy growth of mold whereas the controls were rarely affected. Root hair development was greatly retarded in those seeds which had been dialyzed for the longer periods of time. They were shorter in length and fewer in number.

**SAND AND SOIL CULTURES.**—The same procedure was followed as in the previous experiments. In this case, ten seeds were removed from the dialyzer at the end of the first, second, third, and sixth hours. They were watered with tap water. The results at the end of three weeks closely approximated the results obtained from germinating the seeds in Petri dishes. In addition to the effect on germination, there was a progressive, decreasing gradation in size from the controls to the seeds dialyzed for three hours. All of those dialyzed for six hours were killed (fig. 3).

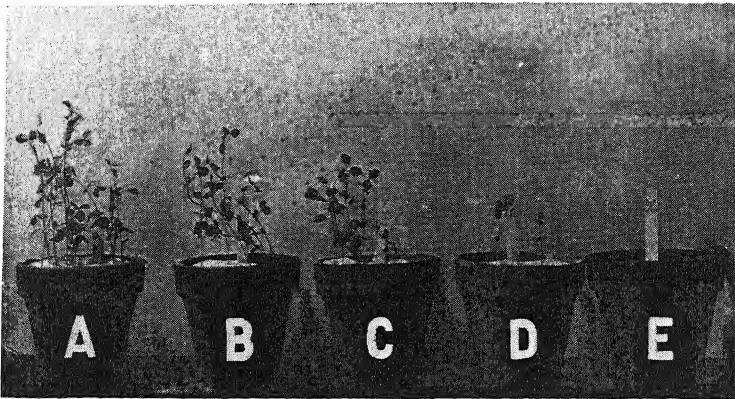


FIG. 3. Effect of electro dialysis on peas grown in sand with tap water; age, 3 weeks. A. Control; soaked 6 hours without dialysis. B. Electro dialyzed 1 hour. C. Electro dialyzed 2 hours. D. Electro dialyzed 3 hours. E. Electro dialyzed 6 hours.

Other seeds were then similarly treated except that they were planted in soil. The percentage of germination was affected as in all the other cases. The rate of development was at first slowed up but after three or four weeks the surviving dialyzed plants apparently recovered entirely and could not be distinguished from the controls.

EFFECTS OF ELECTRODIALYSIS ON DRY WEIGHT AND ASH.—The air-dry weight of lots of seventy-five pea seeds was recorded. Here the procedure differed a little from that followed in the preceding experiments in that all of the seeds of each lot were dialyzed for the same length of time. Various lots were dialyzed for periods of three, four, five, seven, and twelve hours. After

TABLE I  
DRY WEIGHT AND ASH OF ELECTRODIALYZED SEEDS

TIME	TREATMENT	AIR-DRY WEIGHT	OVEN-DRY WEIGHT	ASH WEIGHT	PERCENTAGE ASH*
3 hr.	control	14.343	13.170	0.377	2.63
	dialyzed	14.386	12.870	0.232	1.61
4 hr.	control	14.213	13.143	0.363	2.56
	dialyzed	14.264	13.015	0.220	1.54
5 hr.	control	14.429	13.178	0.344	2.38
	dialyzed	15.389	12.670	0.139	0.914
7 hr.	control	14.366	13.396	0.322	2.24
	dialyzed	15.079	13.094	0.142	0.942†
12 hr.	control	14.491	13.346	0.146	1.09
	dialyzed	15.034	12.282	0.071	0.473

\* Based on air dry weight.

† Reason for this discrepancy not known.

treatment, the seeds were dried at 100° C. and weighed. They were then ashed in platinum crucibles and the ash weight was determined. Results are shown in the following table.

It is evident from table I that a large amount of material may be lost through simply soaking the seeds. At the end of twelve hours of soaking the control seeds had lost about 60 per cent. of their original ash weight. This loss, however, did not affect the viability of the seeds as judged by their germination. The seeds which had been electrodialyzed lost much more material than did the controls by mere soaking. Comparing the electro-dialyzed seeds with the controls on a basis of their ash weight the dialyzed seeds retained approximately 40 to 60 per cent. less ash than did the soaked controls. These figures do not take into consideration the slight differences in the original weight of the two lots of seeds which, however, would not materially change the above values.

### Conclusion

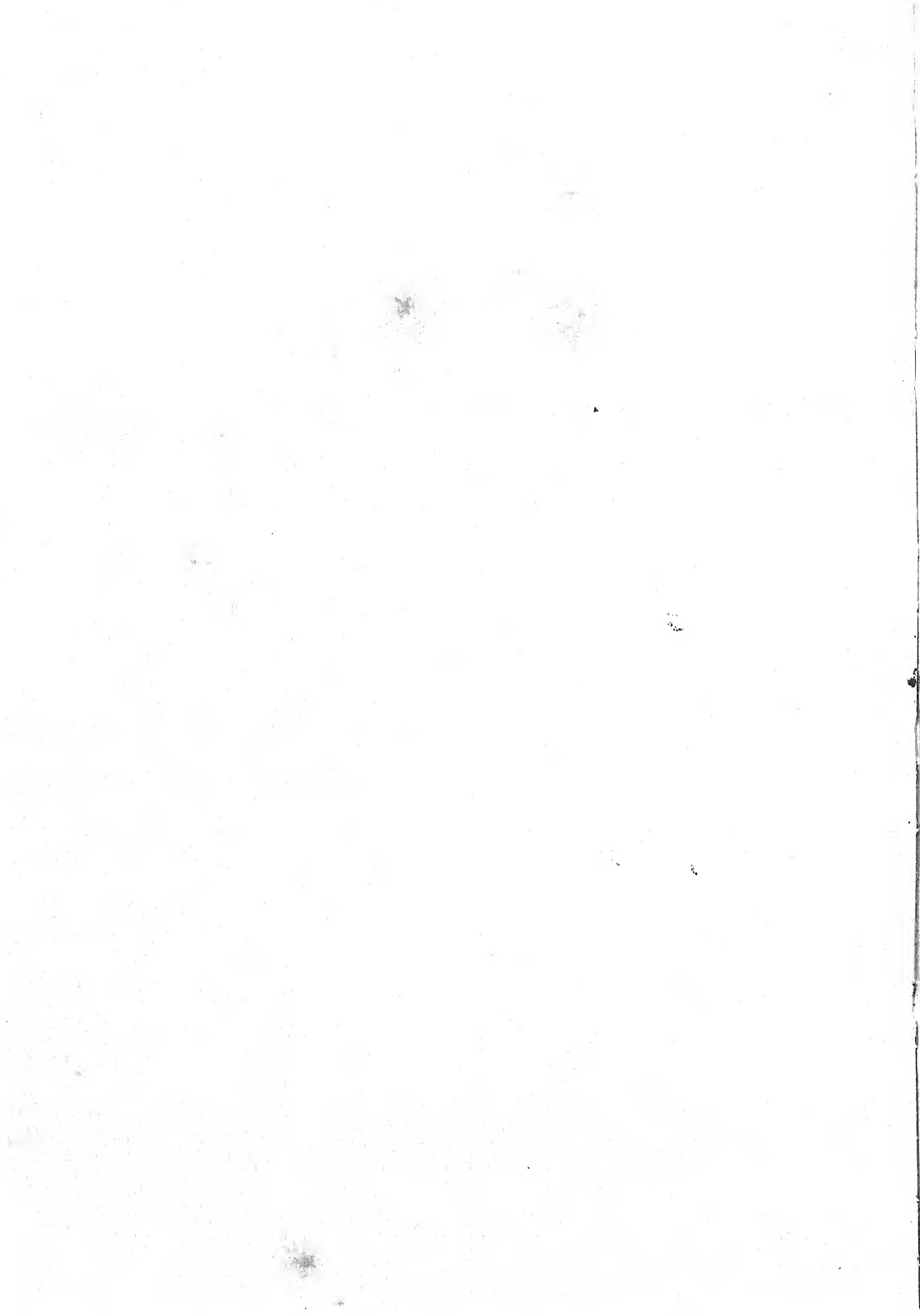
It is evident from the foregoing data that electro dialysis has a detrimental effect upon percentage of germination, resistance to infection, and subsequent development of seedlings. If the treatment is not too long-continued, the plant is apparently able to replace the lost electrolytic material, at least in part, with that obtained from the soil. If the seeds are electrodialyzed for too long a period, they lose their viability.

The writer expresses his appreciation to Dr. C. A. SHULL for his interest in this study.

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# INTERPRETATION OF COMPARATIVE GROWTHS OF FUNGAL COLONIES GROWING ON DIFFERENT SOLID SUBSTRATA

CLAIR L. WORLEY

(WITH TWO FIGURES)

A survey of the available literature in the fields of plant physiology, mycology, and plant pathology clearly indicate that there is a controversy regarding the most accurate method of interpreting the growth extent of a fungous mat on solid nutrient media. Accuracy is particularly important when comparisons are being made of the effects of media with and without various concentrations of growth promoting and growth inhibiting substances. A like controversy has occurred as to the best method to be applied in an effort to interpret accurately the growth curve for a specific mat over a period of several consecutive days.

Two distinct viewpoints have been apparent. The one group holds that in the measurement of colony growth a comparison of the radii is a better criterion of actual growth rate, while the others maintain the ratio of the respective areas is the better. Seldom has a worker seen the desirability of including both types of calculations and of drawing conclusions which would be of significance regardless of the method employed.

In presenting a third method of calculation, it is not the purpose of this paper to magnify this controversy, but rather to present the various techniques and the arguments for and against each.

The various methods and their interrelationships can be derived from figure 1. Figure 1-K represents the control mat and figure 1-T the test mat. In the determination of the growth curves for a given fungous mat, figure 1-T would represent a later stage of growth for the mat of figure 1-K.

The various methods which may be applied to the problem concerned are given in outline form below. All letters included refer to the same letters in figure 1.

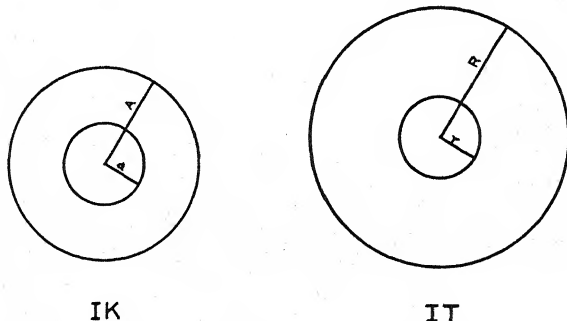


FIG. 1. Diagram of fungal mats. Explanation in text.

TYPE I. Comparison of radii (or diameters) :

- I-a. The length of each radius (or diameter) for the fungous mat on the control and on the test medium is plotted against time, and the several curves are compared.
- I-b. A ratio is set up between the radii of the control and the tests at each measurement. This is the resultant of I-a. The value of the ratio, in all cases, becomes  $R_0 = R/A$ .<sup>1</sup>
- I-c. The increases of radii are plotted as a curve against time. A comparison is then made between the curve for the control and those for the tests.
- I-d. The ratio of the increments of the respective radii claims the greater number of advocates. The value for this ratio is  $DR_0 = (R - r)/(A - a)$ .

TYPE II. Comparison of areas :

- II-a. The total areas for the test mat and the control mat are plotted in reference to time, and the resultant curves are compared.
- II-b. A ratio is established between the total areas; that is, the ratio of  $\pi R^2$  to  $\pi A^2$ . Therefore  $A_0 = R^2/A^2$ , which is equal to  $R_0^2$ .
- II-c. The increases of areas are plotted as a curve against time. A comparison is then made between the curve for the control and those for the tests.
- II-d. The ratio of the increases of the respective areas claims the next largest group of exponents. This ratio becomes  $DA_0 = (\pi R^2 - \pi r^2)/(\pi A^2 - \pi a^2)$ ; that is  $DA_0 = (R^2 - r^2)/(A^2 - a^2)$ .

TYPE III. Apparently, the method offered in this section is new and the arguments in its favor are presented in the following paragraphs. The method takes into consideration the numerous points of growth from the original disk and the area that each covers in its subsequent growth. Theoretically, the points of growth are the growing hyphal tips. If we assume that there are  $n$  such units per millimeter of the perimeter of the inner disk, the area of each "sector" becomes  $S = (\pi R^2 - \pi r^2)/2\pi nr$ , which, in turn, gives the following ratio  $S_0 = \frac{(\pi R^2 - \pi r^2)}{2\pi nr} \times \frac{2\pi na}{(\pi A^2 - \pi a^2)}$ , or  $S_0 = \frac{a(R^2 - r^2)}{r(A^2 - a^2)}$ , or in terms of the above  $S_0 = (a/r)(DA_0)$ .

Figure 2 diagrams that growth which is actually compared by each method of calculation. In the ring-area method, the shaded area in figure 2-A is compared for the control and the test mats. In the radial method the comparisons are based on the relative lengths of line  $X$  for the controls and the tests (*cf.* fig. 2-B). While the new method, the sector-area method, compares the respective areas as portrayed in figure 2-C.

<sup>1</sup> All letters with subzeros refer to ratios; *e.g.*,  $R_0$  is the ratio of the radii,  $DA_0$  is the ratio of the differences of the areas, etc.

It is a well known fact that the actual growth of a fungous colony takes place in all three dimensions; the order of their importance being *Radial* > *Tangential* > *Vertical*.

Those who argue in favor of the ratio in increases of radii ( $DR_0$ ) maintain that radial growth predominates over the others to such an extent that measurements based on this one dimension of growth approximates the true ratio between the actual growths in all three dimensions. They further maintain that the ring-area ( $DA_0$ ) method does not take into consideration the *amount* of fungus contributing to the next increment of growth. Since the radii ratio method is based entirely on linear growth, the amount of con-

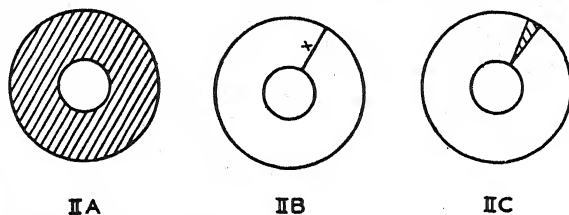


FIG. 2. Diagrams illustrating different methods of comparing fungal growth. Explanation in text.

tributing material plays no part in subsequent calculations. The adoption of the ring-area method assumes that a comparison of a three dimensional growth is approximated more closely by taking into consideration the two more important dimensions than if the primary one (radial) alone is used. The exponents of this method object to the fact that those accepting the other method consider that all growth is linear with none occurring in the other planes. This objection is overrated since after the colonies have attained relatively large diameters, the growth does approach a linear function and growth in the tangential direction is far less important than the ring-area formula would lead one to conclude.

Since the radial method does not, in any case, take into consideration the growth in the second measurable dimension, and since the ring-area method fails to take into account the amount of fungous material present which contributes to this new increment of growth, a ratio-formula was devised in an attempt to overcome both of these objections and yet to incorporate the desirable features of the two older methods. This suggested the sector-area ( $S_0$ ). The number of unit growing points does not necessarily mean the number of hyphal threads per millimeter; if that were true, this method would be nearly ideal and no objection of importance could be raised against its validity. It is to be remembered, however, that no ratio based on only one or two dimensions can be more than an approximation. Any formula, therefore, which gives a result closer to the actual condition (theoretical) is a far better approximation.



Several obvious, interesting, and important relationships can be seen. If the initial growths are identical, the value of  $S_0$  and that of  $DA_0$  are identical. If the conditions arise where the ring-area ratio becomes one, then  $S_0 = a/r$ , which is the reciprocal of  $R_0$ . These and other relationships can be most easily seen by actual calculations based on hypothetical data (cf. the table). All types of measurements given in the table were experienced in actual experiments conducted in the laboratory.

TABLE I  
CALCULATIONS BASED ON HYPOTHETICAL DATA

CASE	a	A	r	R	$DR_0$	$DA_0$	$S_0$
I .....	1	2	1	2.5	1.50	1.75	1.75
II .....	5	7	7	9	1.00	1.33	0.95
III .....	2	3	3	4	1.00	1.40	0.93
IV .....	9	11	11	13	1.00	1.20	0.98
V .....	4	5	3	4	1.00	0.78	0.97
VI .....	6	8	3	5	1.00	0.57	1.14
VII .....	1	4	7	8	0.33	1.00	0.14
VIII .....	2	14	4	20	1.33	2.00	1.00
IX .....	6	9	4	6	0.67	0.44	0.67
X .....	3	9	10	15	0.83	1.74	0.52

Case I shows that the ring-area method gives the same value as the sector-area method when the original radii are identical. Under no other set of conditions is this possible. The only situation under which all three will yield the same factor is when  $a=r$  and  $A=R$ . It is to be noted, however, that these two sets of conditioned measurements are exceptions rather than rules.

In cases II, III, IV, V and VI no effect is shown by the radial method, while either augmentation or inhibition is made evident by either of the other two methods of calculation. The ring-area method shows no effect for the measurements in case VII, while either of the other two types of calculation yield a factor of marked inhibition. Case IX is a special situation under which all three methods suggest marked inhibition. And in case X there is revealed an overemphasis on the tangential dimension by the ring-area method.

If we assume for the present that the sector-area method is the most accurate of the three, we may see the following relationships made evident by the table. (1) If the value of  $S_0$  is 1.00, the other two methods show significant and marked augmentation (VIII). (2) If  $S_0$  gives values showing inhibition, the value of  $DR_0$  may show no effect (II, III, IV or V) or inhibition (VII, IX or X), while the  $DA_0$  factor may suggest augmentation (II, III, IV or X) or no effect (VII) or inhibition (IX). (3) On the other hand, if the factor  $S_0$  indicates augmentation, the  $DR_0$  factor shows augmentation

to a lesser degree (I) or no effect (VI), while the  $DA_0$  factor shows the same effect (I) or marked inhibition (VI). Other variations, although related to those above, may be shown by choosing another set of data.

It can be seen, therefore, that the sector-area method takes into consideration (1) growth in the radial direction; (2) growth in the tangential direction; (3) the number of units contributing to the subsequent growth; (4) the relationships of initial and of total growths, and consequently, of the new increments of growth for the two colonies compared; and (5) the relative importance of radial and tangential growth quantities depending on previous conditions. The radial method fails to consider steps 2, 4, and 5 above. The ring-area method omits steps 3 and 4, and either exaggerates or diminishes markedly step 5.

It is to be remembered that the sector-area method is to be employed only for detecting the effect of a given substrate, as compared to a control, from one time interval to the next and for calculating successive growth rates of a given colony. The total-area method is best adapted for calculating the cumulative effects, while the total-radii, the difference of radii, and the ring-area methods prove to be inadequate for any type of measurement yet made for the growth of a fungal mat on solid nutrient media.

My sincere thanks are extended to Dr. B. M. DUGGAR, of the University of Wisconsin, for his critical review of the protocol.

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# THE CALIBRATION OF A CONSTANT RECORDING ILLUMINOMETER WHEN THE SENSITIVE SAMPLING SURFACE IS HORIZONTAL<sup>1</sup>

DONALD R. WILLARD AND JOHN B. SMITH

(WITH TWO FIGURES)

## Introduction

A recent issue of PLANT PHYSIOLOGY contained a description of a constant recording potentiometer developed by WALLACE (3), who subsequently suggested its use in conjunction with a Weston Photronic cell for recording sunlight for routine weather records (4). Arrangement and use of the apparatus was discussed in considerable detail, but no thorough attempt at calibration in terms of customary light units was reported. By arrangement with Dr. WALLACE, the instrument was purchased, and has been in intermittent use in this laboratory for considerable periods of time. The object of this brief paper is to report an attempt at calibration of the apparatus in foot-candles by comparison with results obtained with a Macbeth illuminometer and a Weston illuminometer.

This empirical comparison indicates the accuracy obtained under the specific conditions described. As described here, the instrument does not provide a complete record of light energy or its effects on plant functions. It does, however, offer possibilities of data for weather records with an accuracy greater than that from mere visual observation or from intermittent readings with light meters.

## Procedure

The recording potentiometer was set with the zero point about eight millimeters from one edge of the paper, at a sensitivity such that the maximum sunlight caused the recorder pen to move about seven inches. It was activated by a Weston Photronic cell, with a ten-ohm shunt across the input terminals of the recorder. This shunt is necessary to secure an approximately linear relationship between foot-candles falling upon the cell and current output over the wide range of light incidence, since, according to the Weston Electrical Instrument Corporation (2) the current output of these cells is much more characteristic of the light intensity than is the voltage generated.

The cell was mounted horizontally on a suitable rubber base, upon a wooden platform built over an unused chimney on the roof of the laboratory. It was covered by an inverted, heavy, white glass, flat-bottomed bowl, which

<sup>1</sup> Published by permission of the Director of Research as Contribution no. 529 of the Rhode Island Agricultural Experiment Station.

rested upon a thick rubber disk. This bowl protected the cell from the weather, absorbed or reflected 75 per cent. of the light falling upon it, and was just large enough to cover the cell. The arrangement of the cell and bowl is shown in figure 1. The bowl was held down level on the rubber disk by suitable clamps, and the interior was connected to a small rubber balloon on the under side of the platform over the chimney. This provided for expansion and contraction of the air under the bowl, as suggested by Prof. C. I. GUNNESS, of Massachusetts State College. A few pieces of anhydrous calcium chloride were placed under the bowl to maintain low humidity.

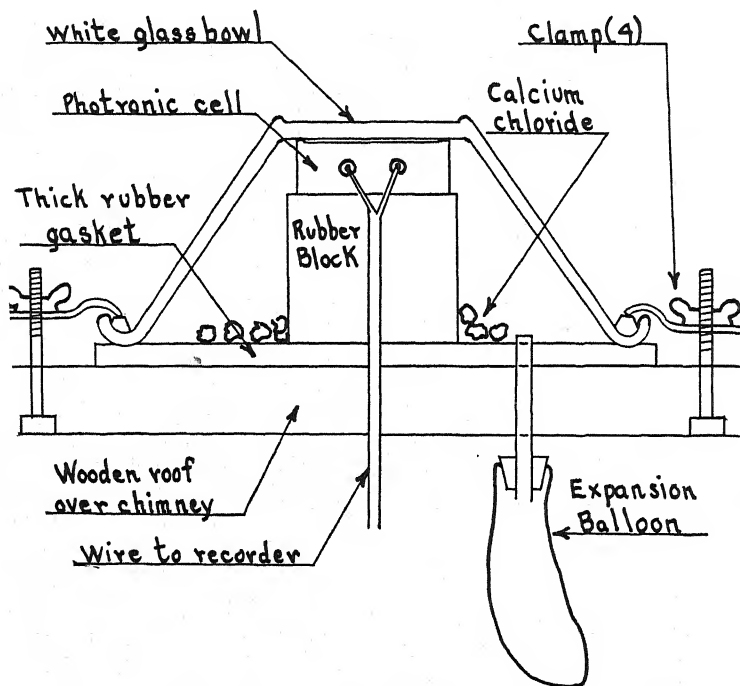


FIG. 1. Photocell assembly on roof.

The calibration was accomplished by means of foot-candle readings taken simultaneously with a Leeds & Northrup Macbeth illuminometer, equipped with a daylight absorbing screen, and a Weston illuminometer from horizontal reflecting surfaces, at stations within fifty feet from the photoelectric cell. The target of the Weston illuminometer was placed in a horizontal position at the same light station. These readings were plotted against the rise of the pen on the drum of the recorder in millimeters. The observations were made at the same time as the foot-candle readings. Actually about two minutes were required for observations, and care was exercised that the light intensity did not change appreciably during this time. Only clear days when the sky was free from clouds were selected.

### Results

Foot-candle readings from both illuminometers were plotted against millimeters rise of pen on drum (fig. 2). This graph shows the agreement which may be expected between the Weston and Macbeth illuminometers. It will be noted that the two instruments agree quite well above 4000 foot-candles, but that below this value the curves separate, and apparently the Weston readings are too low. This may be attributed to the large angle of incidence, measured from the normal, when the sun is low on the horizon, causing reflection from the glass surface over the cell of the Weston illuminometer, and also to the shadow cast by the rim of the case holding the cell. Neither of these factors affects the readings of the Macbeth instrument.

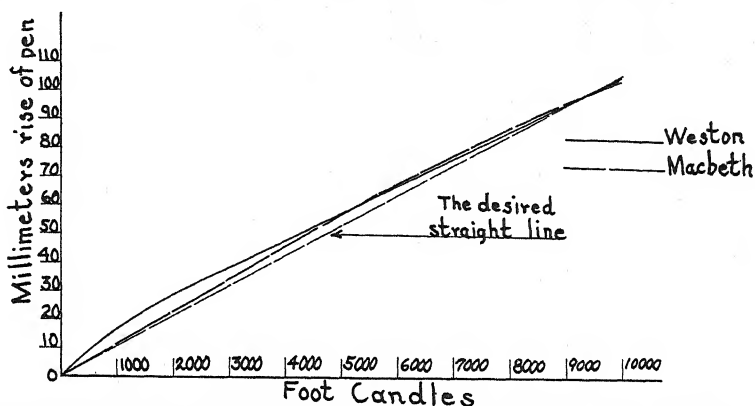


Fig. 2. Comparison between Macbeth and Weston illuminometers, April 30, 1937.

The graph also shows the desired straight line relationship between foot-candles falling on the cell, rise of pen in millimeters from maximum to minimum readings, and the divergence of the actual observations from this desired straight line. The reading of the pen is a little high throughout the entire range, the greatest divergence being at about 4800 foot-candles. This indicates that the relationship between rise of pen, or cell output, and foot-candles is not exactly a straight line. The maximum error is at 4800 foot-candles, where it is approximately 350 foot-candles or 7.3 per cent. The error decreases on both sides of this value.

### Discussion

The resistance across the input terminals of the recorder cannot be reduced much below ten ohms and still have enough potential drop to drive sufficient current through the coil of the galvanometer to move it. Reducing this resistance also reduces sensitivity. The problem becomes one of how much sensitivity can be sacrificed in order to secure as nearly as possible a straight line relationship.

This relationship is important, because it makes possible measurement of the total light upon a surface over a period of time, by integrating the area under the curve drawn by the pen, and expressing the result as foot-candle hours, or in some other convenient unit. A ten-ohm shunt with the thick, white glass, flat-bottomed bowl inverted over the cell, gave better results than several other types of translucent shades that were tried.

Because of changes in the photronic cells, the calibration has not proved permanent, but must be redetermined frequently. Certain of the cells seem much more durable than others. Reduction of sensitivity of a cell may be classed as permanent or temporary. Apparently the cells may be permanently damaged by exposure to the high temperatures of bright summer days. The Weston Electrical Instrument Corporation state that 122° F. (50° C.) is the maximum endurable temperature, beyond which decided changes in sensitivity may be expected. They state also that moisture has a very harmful effect upon the cell (2). These two factors must be guarded against in the assembly of the sampling unit. A temporary failure may be remedied by unscrewing the backs of the cells and inserting small springs, as was done by WALLACE (4) who thoroughly discusses the troublesome weaknesses of the cells. Despite all precautions, however, changes in the relationships between light and the recorded data have occurred.

### Conclusions

With a suitable cell mounted horizontally, and covered with a flat, white glass surface capable of removing 75 per cent. of the total light, the recording illuminometer may be calibrated for light falling on a horizontal surface, using either a Weston or a Macbeth illuminometer as a standard.

The maximum error should not be greatly in excess of 7 per cent. The error means variation of observations from the desired straight line relationship for any cause whatever, and does not consider errors in the Weston or Macbeth instruments. The calibration is not permanent, and must be repeated at intervals.

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## MOLYBDENUM AS AN ESSENTIAL ELEMENT FOR HIGHER PLANTS

D. I. ARNON AND P. R. STOUT

(WITH ONE FIGURE)

The preliminary findings that molybdenum, in minute amounts, improved the growth of barley plants in a culture solution supplied with ammonium salts as the sole source of nitrogen (1), and that a group of seven heavy metals including molybdenum increased the growth of lettuce and asparagus (2), supported the view that the list of essential elements for higher plants is incomplete and suggested the desirability of determining whether or not molybdenum is essential in the nutrition of higher plants.

The essential nature of molybdenum for higher plants was established according to the criteria set up for other elements required by higher plants in minute quantity (3) as follows:

(A) When tomato plants were grown from the seedling stage in rigidly purified nutrient solutions (13) containing all of the eleven nutrient elements now regarded as essential (N, P, K, Ca, Mg, S, Fe, B, Mn, Zn, and Cu) characteristic deficiency symptoms became apparent in a few weeks. The lower leaves developed a distinct mottling different from any other deficiency symptom yet observed in the tomato (fig. 1A). In later stages necrosis at the margins and a characteristic involution of the laminae was evidenced (fig. 1B). Almost all of the blossoms abscised without setting fruit. These deficiency symptoms were produced in six successive experiments at various seasons extending over a period of a year and a half, using both purified and unpurified nutrient solutions; the results, however, were consistent only if the nutrient medium was rigidly purified by procedures efficacious in removing minute metal contaminants (13). Ordinary distilled water and C. P. chemicals contained molybdenum as a contaminant in amounts, adequate at times, to supply plant needs.

(B) The development of these deficiency symptoms was prevented by adding 1 part of molybdenum as molybdic acid to 100,000,000 parts of nutrient solution. This was effective whether added singly or jointly with 19 other elements,<sup>1</sup> none of which were capable of replacing molybdenum [The supplementary microelements' solutions B7 and C13 (2)].

(C) To test the direct effect of molybdenum on the plant as distinguished from its possible effect on the root environment, molybdenum-deficient plants were sprayed with a dilute solution of molybdic acid (0.05 p.p.m. Mo) so as to bring about absorption only through the aerial parts of the plant. Re-

<sup>1</sup> Ti, V, Cr, W, Co, Ni, Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be.



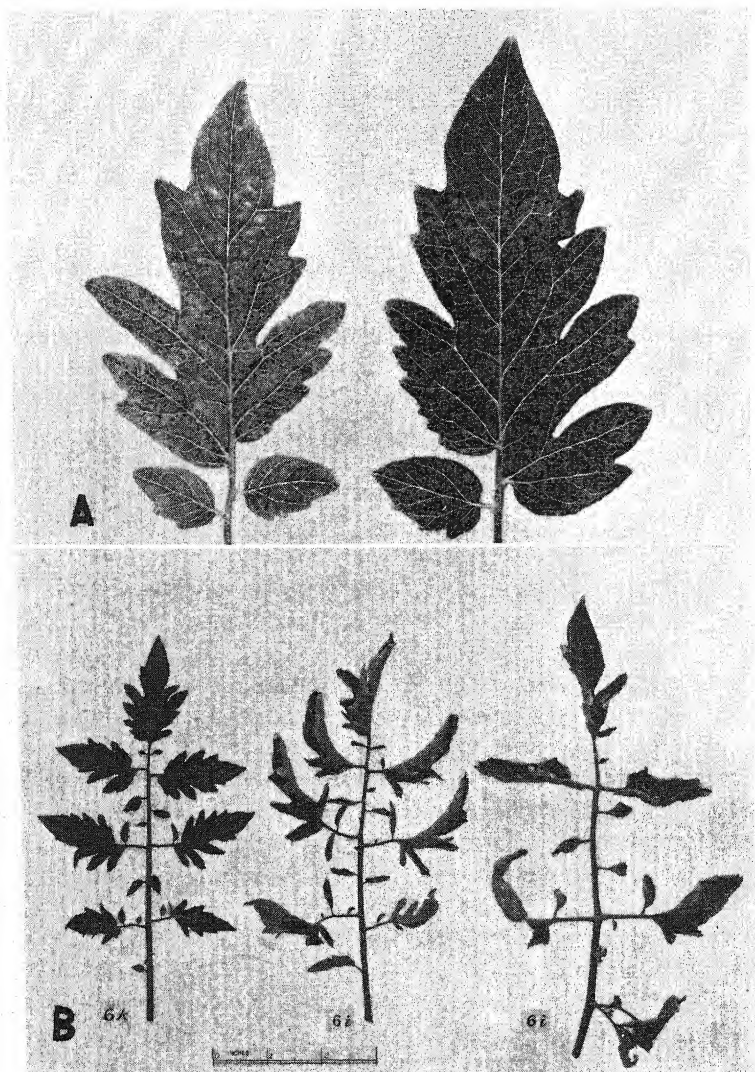


FIG. 1. Molybdenum deficiency symptoms.

- A. Left, mottled appearance of foliage of a molybdenum-deficient plant; right, normal leaflet.
- B. The two leaves on the right show curling and necrosis at the edges characteristic of an advanced stage of molybdenum deficiency; normal leaf at the left.

covery and resumption of normal growth with the disappearance of the molybdenum-deficiency symptoms took place.

Although but 0.01 p.p.m. was required to supply the needs of young tomato plants they tolerated relatively large concentrations of molybdenum

in the nutrient solution, as distinct injury from excess was obtained only if concentrations exceeded 10 p.p.m. (2 mg. of molybdenum per plant).

In recent years the biological importance of molybdenum, particularly with reference to the nutrition of lower plant forms, has been recognized. STEINBERG (11) found that molybdenum is essential for the growth and sporulation of *Aspergillus niger* and that it has a relation, in that organism, to nitrogen metabolism (12). The relation of molybdenum to nitrogen fixation and growth of *Azotobacter* has recently received considerable attention (4, 6, 9). As for higher plants, the widespread distribution of molybdenum has been noted (8, 10), and instances of improved growth as well as toxicity from adding molybdenum to the culture medium, have been reported (5, 14, 7).

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## BRIEF PAPERS

### SINTERED PYREX GLASS AERATORS

GEORGE H. CARROLL

(WITH ONE FIGURE)

Sintered glass aerators have been found to be of great value in water culture experiments. A few years ago PLANT PHYSIOLOGY carried a paper on sintered pyrex glass aerators.<sup>1</sup> This paper, however, did not treat of the actual steps involved in the making of the aerators. The present paper describes a technique for the rapid and economical production of such aerators. A sintered glass aerator is essentially a baked disk of fine glass particles, having sufficient porosity to allow the passage of fine air bubbles; this disk when sealed into a tube constitutes a complete aerator.

The first requirement in the production of an aerator is a quantity of pyrex glass particles of minute size and uniformity, free from impurities. A porcelain ball mill with quartz pebbles will reduce pyrex glass scraps to a fine size when rotated rapidly for a few hours. Particles of uniform size may then be obtained by means of an elutriator, as used by FURNSTAL and JOHNSON,<sup>1</sup> or by sifting through a wire screen, or a piece of silk bolting cloth. By the use of two pieces of cloth, one of 125 mesh per inch and one of 173 mesh per inch, a suitable size may be obtained. By rejecting all particles that do not pass through the larger mesh, and all that pass through the smaller mesh, an appropriate range is obtained. After washing with distilled water and drying, the ground glass is then poured into nickel rings (solid nickel) set on a plate of pure nickel, and the whole baked in a muffle furnace at a temperature of about 780° C. for about 20 minutes. If the furnace is not provided with a thermometer, the temperature may be estimated by observing the color of the heated interior; after a little experience one may safely trust this empirical method. By using a nickel ring cut from tubing of relatively small diameter, 14 mm., a greater number of disks may be accommodated at one time.

There are two ways of assembling the component parts of the aerator, one method requiring special skill in the art of glass blowing; the other, requiring no special skill, yields a product of less presentable appearance, but equally as good in point of service.

In the first method, a short piece of tubing is selected, an 8- to 10-cm. length with an inside diameter about 1 mm. less than the diameter of the sintered disk which is to be sealed in. This difference in diameter is important in facilitating rapid production in the disk-sealing phase. To each end

<sup>1</sup> FURNSTAL, A. H., and JOHNSON, BURKETT. Sintered pyrex glass aerators. *Plant Physiol.* 11: 189-194. 1936.

of the tube is sealed a length of small diameter tubing (see fig. 1, A). This composite piece is then cut exactly in the middle giving two identical pieces. The larger ends of these are then flared slightly to admit a disk. In the process of sintering, a disk acquires a slightly beveled edge, allowing it to fit snugly into the flared end of the tube. The small end of the tube is then passed through a funnel-shaped opening through a block of carbon. A brush from an electric motor (8 cm.  $\times$  5 cm.  $\times$  2 cm.) will do. When the end of the

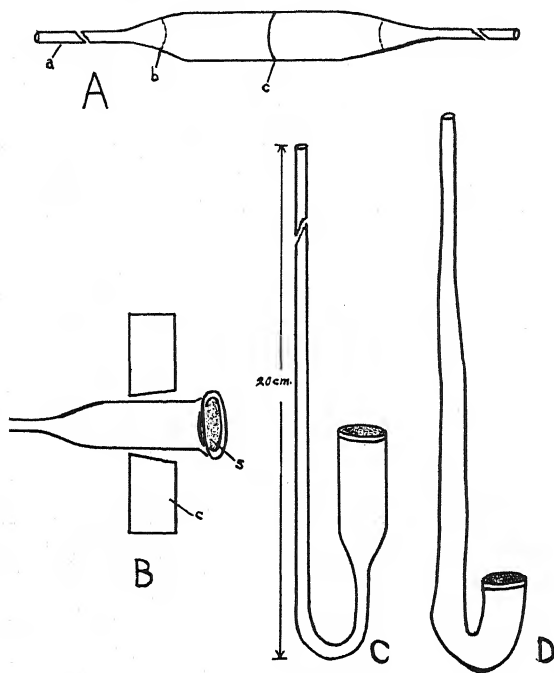


FIG. 1. A. Glass tubing sealed for preparation of two aerators: a, 20 cm. length of 8 mm. tubing; b, junction of 8 mm. and 14 mm. tubing; c, midpoint where cut is made after long pieces are sealed on each end. B. Cross section through carbon block: c, carbon block; s, sintered disk. C. The complete aerator. D. Hand drawn aerator.

tube holding the disk is heated soft, the tube may be drawn through the block and the disk will be firmly sealed in with one operation (see fig. 1, B). The carbon block must, of course, press the soft hot glass against the sintered disk. The junction of the disk and tube must then be annealed. The size of the smaller opening of the funnel-shaped hole through the carbon block must be as great as the diameter of the disk plus the thickness of the tube. An opening of smaller size will cause too great compression and tend to produce an impervious disk. The final step is the bending of the tube to bring the surface of the disk into proper position (see fig. 1, C).

The second method is much simpler than the preceding and is adapted to the tyro glass blower. In this method a single long tube is used, of an inside diameter slightly less (about 1 mm.) than that of the disk. After the disk is sealed in, as in the first method, the tube is heated and drawn out and bent as illustrated in figure 1, D. For this type, a pound of 14-mm. (outside diameter) pyrex tubing when cut to 20 cm. lengths will make about 24 aerators.

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## NITROGEN NUTRITION IN RELATION TO YIELD AND QUALITY OF GRAPEFRUIT

WILLIAM E. MARTIN

Investigations at this and other stations have shown generally that nitrogenous fertilizers exert considerable influence in increasing the yields of grapefruit and other citrus fruits. The factors affecting quality of grapefruit and the relation of nitrogen nutrition to quality have not yet been fully studied with the result that orchard management practices, particularly in Arizona, cannot be made with the assurance of modifying both yield and quality.

A study of the physiology of fruiting of Marsh Seedless grapefruit was begun at this station in the autumn of 1937. Some results have been obtained which may suggest methods of rationalizing the cultural program in terms of treatments affecting both the yield and the commercial quality of the fruit produced.

These experiments indicate that a relatively high nitrogen content of the tissue at the time of blossoming is followed by a relatively large set of fruit. Present evidence suggests that high quality fruit at harvest follows a relatively low nitrogen content of the tree during the summer and fall and may be materially affected by cultural practices carried out during the growing season.

It is suggested that such practices as heavy nitrogen fertilization, with clean cultivation during the summer bring about a vegetative tree condition leading to the production of coarse-textured thick-skinned fruit largely of the lower commercial grades. On the other hand starvation practices such as the growing of competing cover crops, and the withholding of nitrogenous fertilizers during the summer months tend to bring about a low nitrogen composition of the tree, and a condition favorable to the production of smooth-textured, thin-skinned fruit of relatively superior market grades.

Data leading to this view were obtained by grading experimental lots of Marsh Seedless grapefruit at a commercial citrus packing house. The commercial grading of grapefruit is based to a considerable extent upon external appearance. Smooth, symmetrical, thin-skinned fruit are classified into superior grades, while rough, thick-skinned fruit fall into the lower commercial grades or are discarded as culls.

A summary of the experimental treatments, yields, and the proportion of fruit of each of the commercial grades is presented in table I. Yields have been calculated as percentages of the previous year's production.

The accompanying table shows that the trees in plots 1, 2, and 3 which had been given treatments to increase the nitrogen content of the tree prior

TABLE I

EFFECTS OF FERTILIZER AND CULTURAL PRACTICES UPON YIELD AND QUALITY OF MARSH  
SEEDLESS GRAPEFRUIT

PLOT	WINTER TREATMENT	YIELD	SUPPLEMENTARY TREATMENT		QUALITY AT HARVEST			
			SPRING	SUMMER	No. 1 SUN- KIST	No. 2 CHOICE	No. 3 STAND- ARD	No. 4 CULLS
1 .....	High nitrogen fertilization	% 196.8	High N	High N	% 13.7	% 27.8	% 34.5	% 24.0
2 .....		180.9	Starved	Starved	40.1	32.7	13.9	13.3
3 .....		192.8	High N	Starved	32.4	31.4	19.4	16.8
4 .....	Starvation practices	148.8	Starved	Starved	39.9	34.2	11.5	14.4
5 .....		156.3	High N	High N	16.8	31.5	28.6	23.1
6 .....		138.8	Starved	High N	19.4	26.3	26.5	27.8

to bloom, produced about 30 per cent. more fruit than did the trees in plots 4, 5, and 6 which were unfertilized during the winter and had been further starved by a competing cover crop of barley. The analysis of samples taken just before bloom showed much more nitrogen in the leaves and twigs taken from plots 1, 2, and 3 than was found in similar samples taken from plots 4, 5, and 6. Nitrogen fertilization during the winter as well as during the spring and summer was accomplished by the addition of calcium nitrate which is believed to be immediately available to the tree.

Summer treatments appear to exert a marked influence upon the commercial quality of the fruit at harvest. Plots 1, 5, and 6 received nitrogenous fertilizer during the summer and the trees became deep green in color, made flushes of summer growth, and produced a large proportion of culls and a small proportion of first grade fruit. On the other hand, plots 2, 3, and 4 which received no summer nitrogen applications and which were starved by the use of a competitive cover crop of Sudan grass, produced a relatively small proportion of culls and a large proportion of high quality fruit according to commercial grading. Fertilizer applications and starvation practices during the spring months appear to have had similar but lesser effects upon both yield and quality.

If present evidence is borne out by further studies, orchard management programs in Arizona could then be planned by considering the seasonal needs of the tree and nitrogen could be applied when it would do the most good in maintaining production, but be withheld or diverted by cover crops during the season of the year when its presence might impair the commercial quality of the fruit.

DEPARTMENT OF HORTICULTURE  
UNIVERSITY OF ARIZONA  
TUCSON, ARIZONA





## NOTES

**Annual Election.**—The sixteenth annual election of the American Society of Plant Physiologists has resulted in the choice of Dr. JOHN W. SHIVE, New Jersey Agricultural Experiment Station, as president for 1939–1940. The other officers elected are Dr. F. P. CULLINAN, U. S. Department of Agriculture, vice-president; Dr. W. E. LOOMIS, Iowa State College, secretary-treasurer; Dr. D. R. HOAGLAND, University of California, member of the executive committee for three years; and Dr. B. E. LIVINGSTON, member of the board of editors for three years.

With these able leaders another year of progress and development is assured. As the Columbus meeting is approaching, the first duty of all members will be hearty cooperation with the new officers and the program committee in developing an adequate program. All arrangements should be made early to avoid the necessity of last minute decisions. Those who desire to appear upon the general programs should be ready to respond promptly to the call for papers. Arrangement of the papers in non-conflicting groups for parallel programs requires plenty of time for intelligent decisions.

**Summer Meeting.**—The Milwaukee and Madison meeting was the ninth summer meeting of the Society. The convention opened on Tuesday, June 20, with a symposium on photoperiodism at which Dr. A. E. MURNEEK presided. The discussion was opened by Dr. W. F. LOEHWING, who discussed the present general status of the problems of photoperiodic response. The hormone relations were discussed by Dr. K. C. HAMNER, and the anatomical and temperature modifications were stressed by Dr. R. H. ROBERTS. About 45 were in attendance at the opening session.

An informal luncheon for plant physiologists at the Hotel Schroeder was happily arranged by the program committee. On Tuesday afternoon the necessary business was transacted, following which several papers were read. These dealt with ethylene production and the climacteric in apples, carbon dioxide storage of strawberries and raspberries, and the influence of legumes on the nitrogen nutrition of other non-leguminous crops.

The interest shifted to Madison for meetings on Wednesday and Thursday, the University of Wisconsin being host to the general gathering of botanists. President DYKSTRA, and Dean FRED of the University extended welcome to their guests, and responses from the visiting groups expressed the pleasure of the guests at their reception. Wednesday afternoon two invitation papers were presented, one on the rôle of the more abundant mineral elements by G. F. THORNTON, and one on radiation as applied to some current physiological problems, by B. M. DUGGAR. Following these addresses, time was taken to visit Professor DUGGAR's radiation laboratories.

The final meeting on Thursday morning was devoted to a group of miscellaneous papers of general interest. After lunch, inspection of the laboratories for synthetic nitrogen fixation, and the biochemistry laboratory was the chief interest of laboratory men. The field men were personally conducted to the agronomy field experimental plots. At 4:00 P.M. a joint picnic for all groups closed the meeting.

While the attendance was lighter than usual, those who did come felt well repaid, and the committee in charge of arrangements, Dr. W. E. TOTTINGHAM, Dr. I. L. BADWIN, and Dr. L. F. GRABER deserve praise, and sincere thanks for the able handling of the convention arrangements.

**New England Section Meeting.**—The New England Section held its sixth annual meeting at Yale University, May 12–13, 1939. In addition to the presentation of papers concerned with the results of investigations, there was a special meeting for those engaged in blueberry and cranberry research. The session devoted to teaching problems was well attended. It was voted to accept an invitation to meet at Dartmouth College in 1940, with Dr. C. J. LYON, chairman, Dr. R. H. WALLACE, vice chairman, and Dr. L. H. JONES, secretary-treasurer.

**Editorial Service.**—The changes in the editorial service ordered at the Indianapolis meeting in 1937 increased the necessary work and keeping of records very materially. With the doubling in size of our official journal, the editorial responsibilities had grown to such an extent that there was not enough time to take care of all of the required work in a single office. The handling of manuscripts previous to their use interfered with the progress of the journal through the processes of manufacture. Serious delays arose, and these have forced us to adopt certain changes to relieve the pressure upon the editorial functions. Every member of the Society should note these changes, and cooperate with us to render the service most promptly and efficiently.

The receipt and handling of papers during critical examination and revision has been placed in another office. Dr. WALTER F. LOEHWING, Department of Botany, State University of Iowa, Iowa City, Iowa, has consented to serve as secretary to the editorial board. All manuscripts should be sent directly to him. He will send them to readers in whose field the papers fall, communicate with the authors as to advisable revisions, and assume full responsibility for care of the manuscripts until they have been passed upon by the editorial readers. Papers which successfully pass these requirements are then transmitted to the editor-in-chief, presumably to be used, unless there are obvious reasons for some other course. The editorial office will concentrate upon the problems of getting the journal out on time, meticulous editing of the papers, indexing of volumes, and all other matters

concerned with the issuance of the journal, preparation of author reprints, etc. This division of labor, it is hoped, will render the service less burdensome, lead to more prompt and efficient handling of our publication activities, and yet maintain the same standards of excellence for PLANT PHYSIOLOGY. Authors of papers are requested to send their manuscripts directly to Dr. LOEHWING; and please do not ask us to read and advise about papers which are not yet ready to be submitted. There is no time available for such advisory services.

**Chemical Methods Committee.**—The committee on analytical methods is one of the oldest standing committees of the American Society of Plant Physiologists. It was constituted almost at the beginning of our organized work, and for nearly 14 years has served the need for accurate information on analytical methods. The first reports were published in the first volume of PLANT PHYSIOLOGY in 1926, and from time to time additional reports were published, supplemental to the original papers. The most recent contribution of the committee, as yet unpublished, describes the methods of analysis of the main mineral elements found in plant materials. This report is to appear in the October number of the official journal.

Dr. TOTTINGHAM gave unstinting and distinguished service as chairman of this committee throughout the period since the committee was first organized. In accordance with his expressed desire to be relieved of his duties in connection with the committee's work, president LOEHWING undertook a revision of the personnel. As now constituted, the committee chairmanship is held by Dr. W. E. LOOMIS, who has served on the committee since the beginning. Other members holding over are Dr. Z. I. KERTESZ, and Dr. T. G. PHILLIPS. The newly appointed members are Dr. J. T. SULLIVAN, who is physiologist in the regional laboratory for pasture research, U.S.D.A., at the Pennsylvania State College, and Dr. A. G. NORMAN, formerly in charge of the biochemical section at the Rothamsted Experimental Station, now professor of soils at Iowa State College. The committee has made a distinct contribution to physiological research, and will undoubtedly continue to render valuable service in developing better methods, or evaluating older procedures.

**Algae.**—Under the title *Algae, the Grass of Many Waters*, Dr. LEWIS H. TIFFANY, head of the Department of Botany at Northwestern University, presents an interesting and valuable story of fresh water and marine algae. A great deal of the information given in this book will be of interest to physiologists. There are 13 chapters, many of them brief, and the entire work comprises only 171 pages. The chapter titles sufficiently indicate the contents. They are as follows: what are algae? algae and the foods they make; how algae grow and reproduce; algae of lakes and ponds; algae of streams

and rivers; algae of the sea; algae of the soil; algae of ice and snow; algae of bizarre abodes; algae of the past; algae and human welfare; how to collect algae; and how to study algae. A list of general literature references, and an index conclude the volume. Written in delightfully informal style, it appeals very strongly to the student, should make many friends for the science of algology, and for the author. The book is published by Charles C. Thomas, Springfield, Illinois, who quotes the price as \$3.50 per copy. There are more than 50 illustrations.

**Annual Review of Biochemistry.**—Volume VIII of the *Annual Review of Biochemistry* contains twenty-five excellent reviews, most of which are valuable to the plant physiologist. We have come to expect these reviews to be first class summaries of the fields included, and they more than fulfill the expectations. The first review covers the field of biological oxidations and reductions. The dehydrogenases, flavoproteins, mutases, polyphenol oxidases, uricase, dioxymaleic oxidase, catalase, ascorbic acid, citric acid, etc., are given consideration, as well as the unsatisfactory nomenclature, and the problem of potentials. The author, MACOLM DIXON, is himself a contributor in this field; he has handled the review in masterly fashion.

The proteolytic enzymes are reviewed by K. LINDERSTRØM-LANG, and non-proteolytic enzymes by K. MYRBÄCK. A number of the reviews are devoted to metabolism, the chemistry and metabolism of the compounds of sulphur, by G. MEDES; carbohydrate metabolism, by I. L. CHAIKOFF and A. KAPLAN; lipid metabolism, by W. M. SPERRY; metabolism of proteins and amino acids, by R. W. JACKSON and J. P. CHANDLER; mineral metabolism: calcium, magnesium, and phosphorus, by D. M. GREENBERG; metabolism of brain and nerve, by J. H. QUASTEL. Of special value are the reviews on chemical aspects of photosynthesis, by H. GAFFRON; mineral nutrition of plants, by J. W. SHIVE and W. R. ROBBINS; growth hormones of the higher plants, by F. W. WENT; and the biochemistry of yeast, by E. I. FULMER. Space does not permit mention of all of the papers, but they are all conscientiously prepared, and carefully edited. From the beginning of the series the *Annual Review of Biochemistry* has maintained the highest standards of excellence. Volume VIII may be purchased for \$5.00 from Annual Reviews, Inc., Stanford University, California.

**Experiments in Plant Physiology.**—A laboratory manual of plant physiology adapted to American laboratories has long been needed. An attempt to meet this need has been made in the publication of *Experiments in Plant Physiology* by the McGraw-Hill Book Co. The authors are W. E. LOOMIS, Iowa State College, and C. A. SHULL, the University of Chicago. The manual outlines 167 experiments, from which anyone may select experiments of

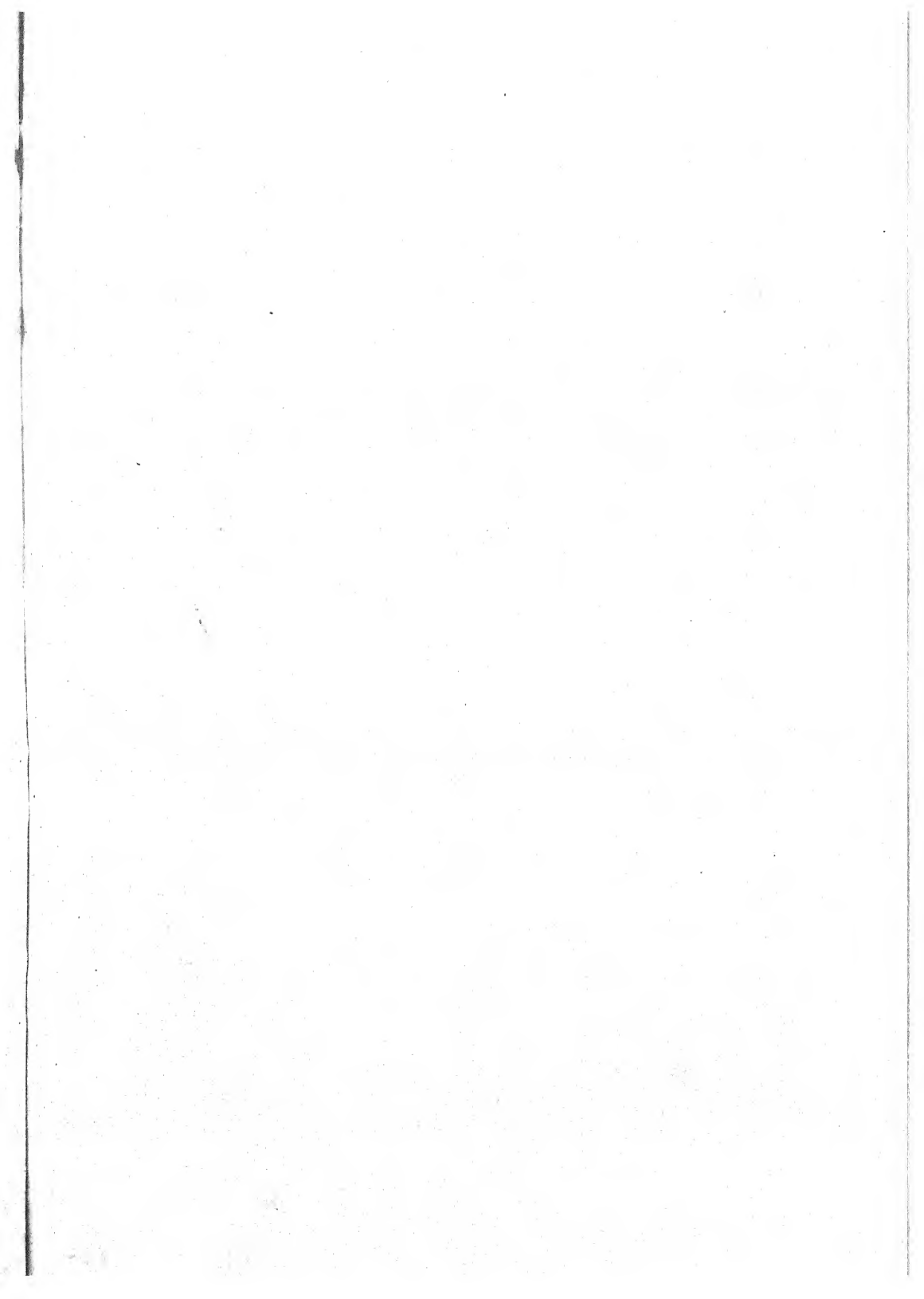
elementary or intermediate grade, and adapt the work to the needs of the students, or to the equipment of the laboratory, or to the time available for instruction, quarter, semester, or year. The price quoted by the publishers is \$2.00 per copy.

**Plant Physiology.**—Another very ably written text has been added to those now available to students of plant physiology. Dr. B. S. MEYER and Dr. D. B. ANDERSON's *Plant Physiology*, published by D. Van Nostrand Co., New York, belongs among the better works devoted to the physiology of plants. The chapters follow an orderly, logical sequence, and the student is prepared to understand the significance of processes by a thorough grounding in the principles of biophysics and colloidal chemistry. The authors are skilful teachers, and have deployed their material in admirable fashion. We hope that every student of plant physiology may read and thoroughly assimilate the information presented. It would probably be impossible to write a text so skilfully that there would not be some criticisms to offer, or differences of opinion as to interpretation. This work, however, is relatively free of flaws, and leaves little to be desired as a statement of current facts and theories in its field. It is a very commendable effort; its thirty-seven chapters are so many fine excursions into the realm of plant functions, and both students and teachers will appreciate the opportunity of using this excellent text. The price is \$4.50 per copy.

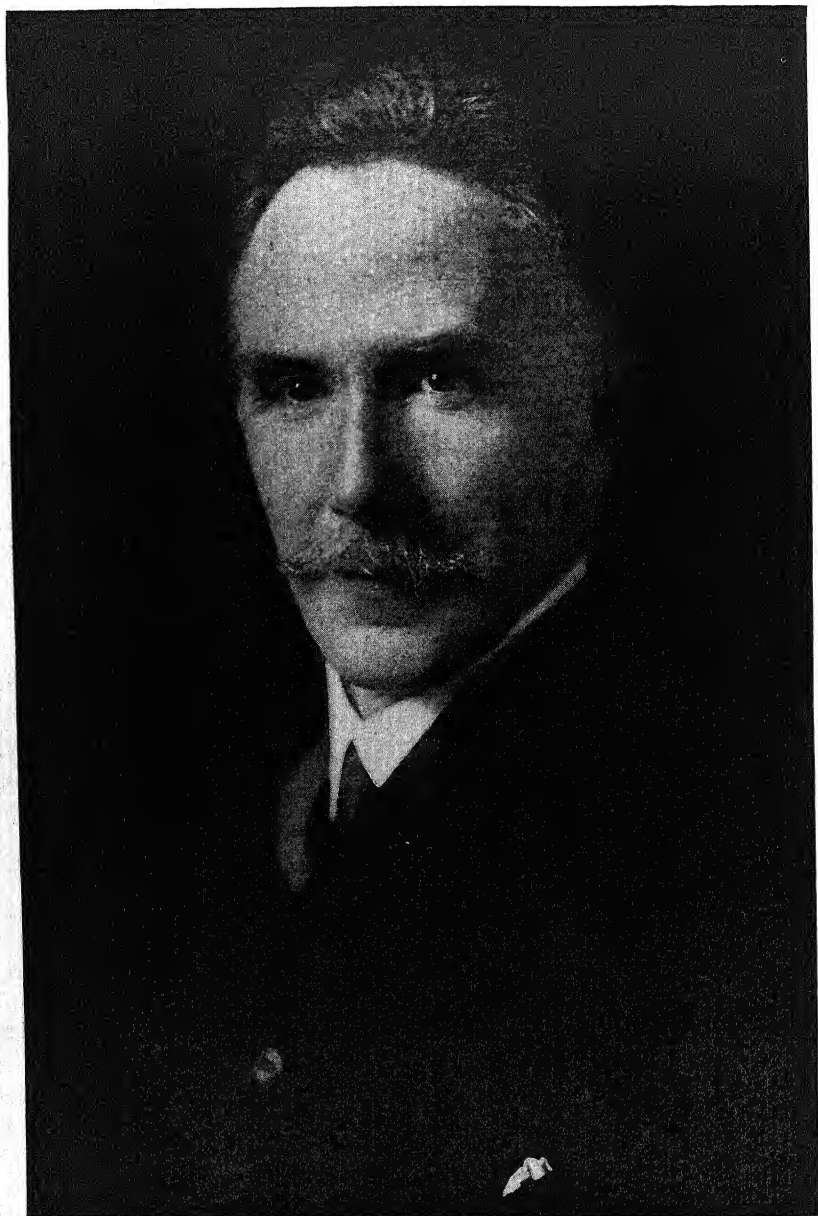
**Elements of Plant Pathology.**—A welcome addition to the books on plant pathology is now available. *Elements of Plant Pathology*, by Dr. I. E. MELHUS and Dr. G. C. KENT, of Iowa State College, is an elementary work, suitable for beginners, and also a work that should appeal to laymen in botanical affairs. It is well written, and fully illustrated (259 figures). The first 7 chapters present the general principles of plant pathology, after which the diseases are treated in systematic fashion, following the classification of the organisms causing disease. The chapters deal with diseases caused by phycomycetes, bacteria, viruses, ascomycetes, fungi imperfecti, basidiomycetes, seed plants, nematodes, and non-parasitic agents. It forms an admirable introduction to the subject, and deserves an enthusiastic reception. The publishers, Macmillan Co., 60 Fifth Ave., New York, offer it to the public at \$4.00 per copy.

**Dictionary of Scientific Terms.**—A third edition of HENDERSON and HENDERSON's *Dictionary of Scientific Terms* has been prepared by J. H. KENNETH. Previous editions were published in 1920, and 1929. A casual examination indicates that more than ten thousand terms are included. There will always be differences of opinion about the adequacy of the definitions

given for the terms included. For instance, the word hormone is defined, for plants, as "internal secretions in plants, as wound or digestive secretions." Would hormone students find the definition acceptable? And should general metabolites be referred to as internal secretions in this case? Such works, however, have a valuable place in the libraries of students and laymen alike. Frequently one encounters terms whose meanings are not suggested by the context, and nothing short of a dictionary suffices in such cases. It is very useful, therefore, to have such a volume handy on one's desk. The publishers are D. Van Nostrand Co., 250 Fourth Ave., New York. The quoted price is \$7.00 per copy. It contains 383 pages, and is unillustrated. Terms are included for Botany, Zoology, Physiology, Anatomy, etc.







HENRY HORATIO DIXON  
MAY 19, 1869

# PLANT PHYSIOLOGY

OCTOBER, 1939

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HENRY HORATIO DIXON

(WITH ONE PLATE)

HENRY HORATIO DIXON was born in Dublin on May 19, 1869, the youngest of seven brothers. The family included also two sisters. The father, GEORGE DIXON, owned a soap works, which is still carried on successfully; one of his brothers was a Fellow of Trinity College. Seldom do seven brothers have such distinguished careers, six in their native city, for in Ireland most families have some members abroad. Two of them carried on their father's enterprise, one was a barrister, one chief inspector of technical schools, and three were university professors, in engineering, anatomy, and botany. Mr. DIXON died while his children were still young, so the burden of upbringing fell on his widow, REBECCA, daughter of GEORGE YEATES of Dublin. She made a home for all and had a welcome for their friends, and later for their pupils. When one considers this large family, so hospitable, so full of interest in many subjects, it becomes clear that in the quiet old lady with her smile of welcome and her lively mind lay the secret of it all.

After attending Rathmines School, HENRY DIXON entered Trinity College, the sole college (founded by Queen Elizabeth in 1591) of the University of Dublin. He obtained a classical scholarship and took prizes in Italian. In 1892 he graduated with first class honours in natural science, which in Dublin meant botany, zoology and geology. He later studied at Bonn under the celebrated STRASBURGER. In 1894 he was appointed assistant to Prof. E. P. WRIGHT and he succeeded to the University Chair of Botany when WRIGHT retired in 1904. WRIGHT had travelled extensively, and his department contained a fine herbarium, with many of his predecessor W. H. HARVEY's type specimens of algae. There was also a beautiful and well stocked botanic garden, so ample opportunity existed for morphological and cytological research. In 1906 DIXON became director of the botanic gardens, and after WRIGHT's death in 1910, of the herbarium also.

No account of DIXON's moulding would be understandable without mention of his friend JOHN JOLY, his senior by some fourteen years. The fam-

ilies were neighbours, and later both moved to adjacent suburban homes. JOLY, the brilliant physicist, found his young neighbour a congenial companion on his yachting cruises. The benefit to a botanist of being able to discuss physiological problems with a competent physicist, was of course great. Thus the names JOLY and DIXON were linked for ever as the originators of the cohesion theory of the ascent of sap. In many university and other activities their names were also associated, and an ideal friendship of fifty years was terminated in 1934 by JOLY's death. To his friend he left his house, his pictures, his books, and it was upon DIXON that the Royal Society of London called to write an outline of JOLY's life.

In 1907 Prof. DIXON married DOROTHEA MARY, daughter of the late Sir JOHN H. FRANKS, C.B., and they have three sons. The eldest, G. JOLY DIXON, is a Member of the Royal College of Physicians at the Charing Cross Hospital, London; the second, Dr. KENDAL C. DIXON, is a Fellow of Kings College, Cambridge; the youngest is still at school. In their own home Prof. and Mrs. DIXON have continued the hospitable tradition of the family.

Outside the university, DIXON's activities have been largely connected with the Royal Dublin Society, which fosters scientific research, agriculture, music, distributes radium emanation, and runs the famous Dublin Horse Show. Of this Society he is a vice-president. He is also on the Committee of the Imperial Bureau of Mycology, London, and is a Commissioner of Irish Lights. The latter involves a yearly inspection of light houses and light-ships, a service still maintained by the British Government. To be hauled up a light house and lowered again into a small boat in the grip of Atlantic rollers is no nominal undertaking.

In 1908 DIXON was elected to the Fellowship of the Royal Society of London, and in 1917 he received the Boyle Medal of the Royal Dublin Society. In 1927 he went to the U. S. A. as visiting professor of the University of California. The beauty of the country, the vast scale of the university, the keen scientific discussions, and the warmth of the personal welcome made a deep impression upon him. In this tour he was accompanied by Mrs. DIXON and it was marked in their family history by the christening of their youngest son as HENRY BERKELEY, born in 1928.

In 1932 DIXON was elected a Corresponding Member of the Botanical Society of America, an honour which brought him pleasant memories of his American friends.

When he reached seventy, this year, DIXON's Irish pupils decided that his birthday, May 19th, should be signalized by a congratulatory address. The suggestion received a ready response and, including some foreign lists which unavoidably came in late, an address with about 600 signatures was presented to him by the Provost of Trinity College at an informal gathering. This testimony to his work, from so many friends and fellow workers in so

many lands, gave him very genuine pleasure. The members of the University also were most appreciative of the honour paid to their professor from abroad.

A list of DIXON's publications may be found in the account of the award of the Boyle Medal, in the Scientific Proceedings of the Royal Dublin Society, 1917, vol. 15, pp. 179-184, obtainable separately as publication no. 19 of the volume. This list of 61 titles includes *Transpiration and the Ascent of Sap in Plants*, Macmillan, London, 1914. In 1924 DIXON delivered three lectures on *The Transpiration Stream* before the University of London. These were published by the University Press. Recently his Royal Society Croonian Lecture entitled *Transport of Substances in Plants* reviewed the subject in all its aspects. It is reprinted in Notes from the Botanical School of Trinity College, Dublin, vol. 4, no. 6, November, 1938. Most of DIXON's papers, which have chiefly appeared in Sci. Proc. Roy. Dublin Soc., have been reprinted in the Notes, which was started in 1896, by Prof. E. P. WRIGHT. Since the Boyle Medal award list, twenty-one additional papers have been re-printed in the Notes.

When DIXON was appointed to the Chair of Botany the department consisted of a few rooms in a residential house in the Front Square, with the herbarium in large rooms on the top. Lectures to the classes of medical students and practical classes were held elsewhere. Through the generosity of Lord Iveagh and a number of graduates of the University of Dublin a new School of Botany was erected in 1907. It bears throughout marks of the forethought of the Professor. No building ever had less space wasted in passages, etc., or rendered useless by inadequate lighting.

The elementary lectures are always delivered by the Professor; each is followed by a practical class in which his assistants and demonstrators teach under his supervision. The class also visits the Gardens. This course was published in 1922 as *Practical Plant Biology* (Longmans, Green & Co.). Starting with the use of the microscope, it deals first with the structure and physiology of unicellular plants and bacteria, and simple cultures are made. The medical student is thus, at the start of his career, brought to understand the meaning of infection and sterilization. Special attention is paid to the real understanding of the sizes of objects seen under the microscope, measurements being made in three dimensions. Types of algae and fungi are then considered, followed by the liverworts, ferns, gymnosperms, and angiosperms. The whole course is constructed to illustrate evolution, and the final lectures deal with this, nuclear division, and theories of heredity. Thus one does not wander from the green leaf, root, and flower back to the unicellular organisms, but progresses mentally in the same direction as was followed in plant evolution and is followed in its life history by every plant. This course is recognised by past students, especially those of the medical

profession, as constituting a landmark in their education; and its creation must be reckoned as DIXON's greatest success as a teacher. Those taking natural science for a degree specialize, in the latter half of the four years course, in one subject. The very limited numbers insure that each student receives that personal contact which can prove so stimulating; and old students who return have the pleasure of realizing that the courses are undergoing a ceaseless revision, and are being illustrated by new lantern slides or by the professor's new lecture experiments or beautiful cytological preparations.

Though not a systematist, DIXON is fully aware of the importance of this branch of botany. Consequently, soon after WRIGHT's death he succeeded in having an adequate addition made to the east end of the School; so that by 1912 the Herbarium was housed better than ever before, in a well lighted room, together with many floristic works. The labour of moving, sorting, and treating hundreds of bundles to destroy insects was immense, and was carried out with but little assistance.

The duties of Director of the Botanic Gardens have not been interpreted by DIXON as a sinecure. The beautiful gardens, about a mile from the College, were walled in early in 1807, and in the spring of 1808 the planting of trees, shrubs, and herbaceous plants commenced. They were thus old and well stocked when DIXON took charge, yet he has improved them and added over seven thousand species. One notable addition may be mentioned, a collection of Siamese orchids made over a number of years by A. F. G. KERR, Esq., M.D.

One thinks of DIXON as a physiologist, but in his earlier years he made important contributions to cytology; these include work on the chromosomes and the first mitosis of the spore-mother-cells of *Lilium* and on the nuclei of the endosperm of *Fritillaria imperialis*. He also studied the resistance of seeds to high and low temperatures and to poisons, including radium emanation. His early experience when studying the temperature of the underground organs of plants led to his thermoelectric method of cryoscopy, used in so much of his later work on osmotic pressure. As a memory of his love of sailing we have his Royal Society paper on the structure of Cocco-spheres and the origin of Cocoliths (1900).

During the war DIXON undertook the examination of over forty varieties of wood known commercially as mahogany. This paper, with 138 plate figures, was of technical interest since mahogany was used for aeroplane propellers.

In 1922 DIXON was President of the Botanical Section of the British Association; his address dealt with the vexed question of the transport of organic substances in plants, a problem soon to be greatly clarified by the work of his pupil T. G. MASON. With his assistant N. G. BALL, DIXON

then published an account of the channels of transport from the storage organs of seedlings of *Lodoicea*, *Phoenix*, and *Vicia*. No account of the subject should omit consideration of this paper, in which transport of carbohydrates is considered in an organ where the transport of water is unnecessary.

DIXON's work on variation in the permeability of leaf-cells appeared in 1924. Later, with T. A. BENNET-CLARK, he studied the responses of plant tissues to electric currents and the influence of temperature. This led to an investigation of the electrical properties of oil-water emulsions in relation to the structure of the plasmatic membrane. By 1932 highly important conclusions had resulted from this investigation.

In 1933 DIXON published a paper on bast sap, in which the theory of MÜNCH was specially considered, and he subsequently made an extensive study of the convection of heat and materials in the stem of a tree. In 1938 a paper on subaqueous transpiration appeared, and his 70th birthday found DIXON still busy with his researches, and with his junior class of one hundred.



# RESPIRATION OF ACORNS AS RELATED TO TEMPERATURE AND AFTER-RIPENING<sup>1</sup>

JAMES W. BROWN

(WITH TEN FIGURES)

## Introduction

It is well known that while acorns of the white oak group will germinate as soon as they fall from the tree, acorns of the red oak group will not germinate until they have undergone a period of after-ripening. Although the red oak acorns are morphologically mature they must undergo certain physiological changes before they will germinate even under the most favorable conditions. The nature of the physiological changes occurring during the after-ripening of acorns is not fully understood. KORSTIAN (6) found that after-ripening is affected by temperature. Storage at an average night temperature of 50° F. (10.0° C.) and day temperature of 65° F. (18.3° C.) resulted in the most satisfactory after-ripening as judged by promptness of germination and the percentage finally germinating.

It appears that certain chemical and physiological changes occur in red oak acorns during over-wintering and the accompanying after-ripening which do not occur in acorns of the white oak group. It was believed that concurrent measurements of the O<sub>2</sub> consumption and CO<sub>2</sub> production of acorns of the white and red oak groups might supply some information concerning differences in the physiology of the two groups. It also seemed possible that periodic measurements of the rate of respiration and the respiratory quotient of red oak acorns stored at various temperatures would indicate the effects of storage temperature on the rate of after-ripening and the accompanying physiological changes.

Acorns of northern red oak [*Quercus borealis* var. *maxima* (Marsh.) Ashe] and white oak (*Quercus alba* L.) were chosen for these studies because they are widely distributed and representative species of the two principal groups of oaks.

## Methods

### TREATMENT OF MATERIAL PREPARATORY TO MEASUREMENTS

About 2750 white oak acorns and 2500 northern red oak acorns were collected at the time they fell from the trees in the autumn of 1936 and an equal number was collected in the autumn of 1937. When the last collection was made in late October of 1936, 250 acorns of each species were planted in a greenhouse in a mixture of equal parts of sand and peat. The

<sup>1</sup> This investigation was supported by a Fellowship in Forestry in the Graduate School of Arts and Sciences of Duke University.



remainder of the acorns of both species was placed in the same mixture in flats. The northern red oak acorns were divided into groups and stored at 0°, 10°, and 20° C. The white oak acorns were placed in a cold room at approximately 0° C. These temperatures varied 2° to 3° C. except the storage temperature of 10° which varied only 0.5° C. After six weeks of storage at 0° C., repairs to the cold room necessitated removal of the material to a refrigerator at 2° which varied about 0.5° C.

In 1937, 100 northern red oak acorns and 200 white oak acorns were planted in a mixture of sand and peat in a greenhouse on the day of collection. The white oak acorns to be used in other studies were placed in the sand-peat mixture in flats (KORSTIAN, 7) and stored at 2.5° C. The northern red oak acorns were divided into four groups for storage in the same manner at 2.5°, 12.5°, 15.0°, and 17.5° C. Refrigerators were set at 10° and 15° C. and electric ovens were placed in them to obtain the 12.5° and 17.5° C. temperatures.

In 1936 from 30 to 36 samples were used in a series of determinations, in 1937 only 10 to 18 samples. Each sample consisted of two or three acorns, the number depending on the size. Statistical studies of the 1936 data showed that the smaller number of samples could be used without any appreciable loss in significance of the results. Enough sound acorns to supply the required samples were removed from storage the night before a series of determinations were to be made. The pericarp and any loose part of the testa were removed, the seeds were divided into groups constituting three or four samples, depending upon the conditions of the experiment, then placed in cheesecloth bags, submerged in water, drained a moment and placed in two-quart glass jars. The jars had inner supports of glass and wire about two inches above the level of 150 ml. of 2N NaOH. After the seeds had been placed in the jars, the tops were screwed on and the seeds were kept at the temperature of the experiment until the next day in an atmosphere free of CO<sub>2</sub>. When runs were about to be made, the material was removed from storage, one jar at a time, and transferred from the jar to containers in the bath at the same temperature. These containers also had a supply of 2N NaOH to preserve a CO<sub>2</sub>-free atmosphere. WILLAMAN and BEAUMONT (12) working with apple twigs and wheat in closed containers found that material previously exposed to a high concentration of CO<sub>2</sub> showed an abnormally high CO<sub>2</sub> production when the CO<sub>2</sub> content of the surrounding atmosphere was decreased. This was explained as possibly resulting from CO<sub>2</sub> accumulated in the tissues diffusing out until equilibrium was attained with the surrounding atmosphere. Since, in this study, the determinations of CO<sub>2</sub> production were made in a CO<sub>2</sub>-free atmosphere, storage in such an atmosphere resulted in the acorns already being in equilibrium when the respiration determinations were started. At the appropriate time,

each sample was transferred to a respirometer vessel and measurements of respiration were made. The samples were removed and their volumes determined to the nearest 0.1 ml. by direct displacement in water in a specially constructed tube (RUBINSTEIN, 10).

After the volumes of the samples had been recorded, they were placed in weighing bottles which had been brought to constant weight, set in a drying oven, and kept at approximately 103° C. for 24 hours. The samples were then cooled in a desiccator and weighed. The data were then converted into cubic millimeters of gas per gram of dry weight of the acorns.

In those experiments where respiration was measured at a temperature differing from the storage temperature, the material was kept at the experimental temperature for a period of at least 12 hours before making measurements. Preliminary runs made in the spring of 1936 indicated that it took acorns about 6 hours to approach equilibrium after undergoing a change in temperature (fig. 1).

The acorns of both species of oak were ground immediately upon removal from storage for one set of catalase measurements (fig. 8A and table VII) while the acorns were allowed a 24-hour germinating period at 25° C. for another set of measurements (fig. 8B). All catalase measurements were made at 25° C.

#### RESPIRATION

Warburg respirometers were used to measure the concurrent O<sub>2</sub> absorption and CO<sub>2</sub> production of the acorns in this investigation. The respirometers were immersed in a water bath similar to that described by STIER and CROZIER (11). The bath had a temperature range of from -5° to 35° C. and the total variation at a set temperature was 0.054° C. as measured by a Beckman thermometer.

The principle of the Warburg method is based upon the manometric measurement of changes in gas pressure in a closed system adjustable to constant volume. By placing an alkali in the bottom of the respirometer vessel, it is possible to absorb the CO<sub>2</sub> produced in respiration so that any pressure change shown by the manometer is caused by the consumption of O<sub>2</sub>. After a measurement of O<sub>2</sub> absorption has been made, a reading of the CO<sub>2</sub> produced can be obtained by introducing an excess of acid into the alkali from a side arm of the vessel, thus liberating the CO<sub>2</sub> previously absorbed. The details of this method as applied to plant material have been described by BROWN (4).

#### CATALASE

The technique and apparatus described by LOOMIS and SHULL (8) were employed with certain modifications in studying the catalase activity of the two species of acorns. Two or three acorns were ground with quartz sand

for three minutes and made up to a 10 per cent. suspension by the addition of distilled water. From this suspension 5 samples of 4 ml. each were drawn. A 4-ml. sample was placed in one arm of a catalase tube while 4 ml. of  $\text{H}_2\text{O}_2$  were placed in the other arm. The tube was attached to the apparatus, brought to the temperature of the bath ( $25^\circ \text{C.}$ ), the manometer levelled and read. A check or control measurement for any change in concentration of the  $\text{H}_2\text{O}_2$  during the progress of the experiment was made by determining the volume of  $\text{O}_2$  produced by 2 ml. of  $\text{H}_2\text{O}_2$  and 0.5 gm. of  $\text{MnO}_2$ . The mean of 4 consecutive measurements was obtained and adjusted to 1 gm. of  $\text{MnO}_2$ . The means obtained were all approximately 50 ml. of  $\text{O}_2$  so each was adjusted to 50 ml. The mean of 5 catalase measurements on the acorn suspension was determined and adjusted for variation in concentration of  $\text{H}_2\text{O}_2$  by the factor obtained from the control determination.

### Experiments and results

#### TIME REQUIRED TO ADJUST ACORNS TO A NEW TEMPERATURE

An investigation was made in the spring of 1936 to determine how long acorns used in respiration studies must be kept at a new temperature in order to insure a constant rate of respiratory activity. Northern red oak acorns were taken from storage at  $10^\circ \text{C.}$  and their respiration measured at two-hour intervals for a 10-hour period at  $20^\circ \text{C.}$ , using the original samples for all measurements. Figure 1 shows that a constant rate of respiration

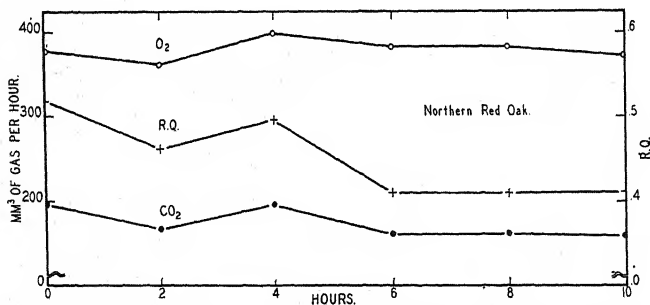


FIG. 1. The time required for northern red oak acorns to attain a constant rate of respiration at a new temperature.

was reached by these acorns in 6 hours. Each point on the graph was determined by the means of 5 samples, except the points at 10 hours, for which only 4 samples were available. It is probable that some physiological adjustment always occurs when material is moved from one temperature to another, and time should be allowed for the attainment of a new equilibrium before making any determinations of respiration at the new temperature. It is also probable that a much longer adjustment period would be needed if the pericarps were not removed from the acorns.

## COMPARISON OF RESPIRATION OF THE ACORNS OF TWO SPECIES OF OAK

Northern red oak and white oak acorns were stored at 0° C. at the time of fall from trees in 1936, and measurements were made of simultaneous O<sub>2</sub> consumption and CO<sub>2</sub> production at intervals over a period of a month during the after-ripening period of the northern red oak acorns. When the acorns fell from the trees in 1937, a similar experiment was started using a storage temperature at 2.5° C. but was continued for two and one-half months.

As shown in figure 2, the respiratory quotients of the two species are very similar indicating that the metabolism of the two is similar. The rate of O<sub>2</sub> consumption of the white oak acorns was from 1.3 to 1.7 times greater

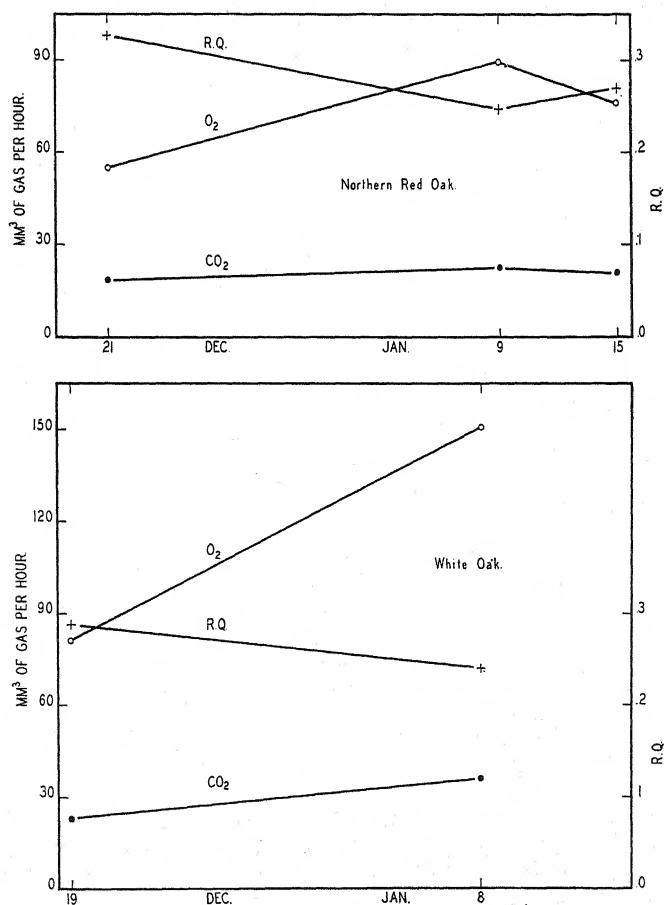


FIG. 2. The difference in respiratory activity of northern red oak and white oak acorns stored and measured at 0° C. in 1936-1937.

than that of the northern red oak acorns. The  $O_2$  consumption of both species increased with length of time in storage while the  $CO_2$  production remained almost constant. From the 9th to the 15th of January the rate of respiration of the northern red oak acorns decreased slightly while the R. Q. remained relatively constant (table I). Measurements of white oak acorns

TABLE I

DIFFERENCE IN THE RESPIRATORY ACTIVITY OF NORTHERN RED OAK AND WHITE OAK ACORNS STORED AND MEASURED AT  $0^\circ C$ .

DATE	GAS	NUMBER OF OBSER- VATIONS	MEAN VOLUME OF GAS PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT ( $R. Q. = \frac{\text{mean CO}_2}{\text{mean O}_2}$ )
Northern red oak						
12-21-36	CO <sub>2</sub>	34	mm. <sup>3</sup> 18.09	mm. <sup>3</sup> 1.20	% 6.66	0.3269
	O <sub>2</sub>	34	55.34	4.90	8.85	
1- 9-37	CO <sub>2</sub>	36	22.14	1.57	7.09	0.2472
	O <sub>2</sub>	36	89.56	6.03	6.73	
1-15-37	CO <sub>2</sub>	31	20.50	1.69	8.23	0.2693
	O <sub>2</sub>	29	76.12	4.38	5.75	
White oak						
12-19-36	CO <sub>2</sub>	36	23.38	1.63	6.96	0.2878
	O <sub>2</sub>	36	81.24	6.72	8.27	
1- 8-37	CO <sub>2</sub>	35	36.20	2.70	7.46	0.2402
	O <sub>2</sub>	35	150.72	5.21	6.91	

were discontinued after the 8th of January because their radicles were so long that it was impossible to place them in respirometer vessels without injury.

Referring to figure 3 and table II, the  $CO_2$  production of white oak acorns was constant during the period of storage at  $2.5^\circ C$ , while the  $CO_2$  production of the northern red oak acorns varied slightly. The  $O_2$  consumption of the northern red oak acorns increased rather sharply from the start of the experiment while the white oak acorns showed a constant rate of  $O_2$  consumption during the first three weeks of storage, a sharp rise during the next three weeks, and a slight decrease in the last three weeks. The R. Q. of the white oak acorns varied inversely as the  $O_2$  consumption since the  $CO_2$  production was constant. The R. Q. of the northern red oak acorns approached very closely that of the white oak acorns during the last 6 weeks of the experiment. There was a difference of about 0.1 in the R. Q.'s of both species during this period.

TABLE II  
RESPIRATION OF NORTHERN RED OAK AND WHITE OAK ACORNS STORED AND  
MEASURED AT 2.5° C.

DATE	GAS	NUMBER OF OBSER- VATIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
Northern red oak						
11-21-37	CO <sub>2</sub>	18	mm. <sup>3</sup> 29.94	mm. <sup>3</sup> 3.62	% 12.09	0.4990
	O <sub>2</sub>	18	60.00	5.20	8.67	
12-16-37	CO <sub>2</sub>	10	9.86	1.94	19.68	0.0613
	O <sub>2</sub>	10	160.88	11.98	7.45	
1- 7-38	CO <sub>2</sub>	10	17.58	3.30	18.77	0.0871
	O <sub>2</sub>	10	201.74	14.22	7.05	
White oak						
10-31-37	CO <sub>2</sub>	18	26.30	2.60	9.89	0.4125
	O <sub>2</sub>	18	63.76	1.32	2.07	
11-21-37	CO <sub>2</sub>	18	30.74	3.46	11.26	0.4910
	O <sub>2</sub>	18	62.60	6.00	9.58	
12-16-37	CO <sub>2</sub>	10	28.42	4.06	14.29	0.1636
	O <sub>2</sub>	10	173.68	9.72	5.60	
1- 7-38	CO <sub>2</sub>	10	25.32	1.46	5.77	0.1594
	O <sub>2</sub>	10	158.82	9.04	5.69	

#### EFFECTS OF TEMPERATURE ON RESPIRATION OF ACORNS

The effect of temperature on the rate of respiration of northern red oak acorns stored at 0° and 10° C. in 1936-1937 was found by measuring their respiration at 0°, 5°, 10°, 15°, 20°, 24° and 30° C. The experiment was repeated in 1937-1938 using both northern red oak acorns and white oak acorns which had been stored at 2.5° C. The respiration of both species was measured at 2.5°, 5°, 10°, 15°, 20°, 25° and 30° C.

Figure 4A shows that the CO<sub>2</sub> production of northern red oak acorns increased along a practically straight line from 5° to 30° C. The O<sub>2</sub> consumption was much less regular in its behavior but generally increased with temperature. The lowest respiratory activity was found at 5° C. The R. Q. of this species increased gradually at the start and more rapidly as the temperature increased from 0° to 24° C., where it reached its maximum of 0.6208 (table III). From this maximum the R. Q. fell to 0.5442 at 30° C.

It should be noted that, in this experiment, the storage temperature for the material measured at 0° and 5° was 0° C. while the material measured from 10° to 30° was stored at 10° C. Lack of material prevented making measurements over the entire temperature range of acorns stored at one temperature.

The respiratory behavior of northern red oak acorns stored at 2.5° and measured over a temperature range from 2.5° to 30° C. is somewhat difficult to explain. The  $\text{CO}_2$  production (fig. 5) was essentially the same as in the previous year (fig. 4A) but did not proceed at as great a rate. The  $\text{O}_2$  consumption generally decreased with increased temperatures where previously it had generally increased. The R. Q., however, although irregular at first,

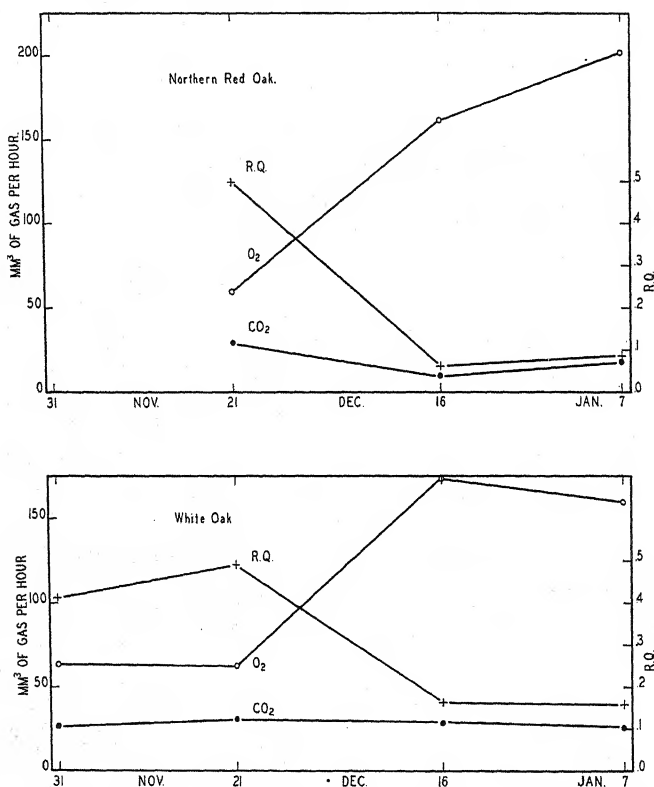


FIG. 3. The respiratory activity of northern red oak and white oak acorns stored and measured at 2.5° C. in 1937-1938.

finally increased regularly with temperature at the higher temperatures (table IV). The R. Q. (fig. 5) of the northern red oak acorns was less than the R. Q. of the preceding year by about 0.2. The material was measured after about the same length of storage both years.

The R. Q. and  $\text{CO}_2$  production of the white oak acorns in 1937-1939 (fig. 5) increased regularly while the  $\text{O}_2$  consumption varied until it apparently reached an equilibrium at 15° which was held until 25°, and then rose at 30° C. The rate of respiration was generally higher in the white oak acorns than it was in the northern red oak acorns.

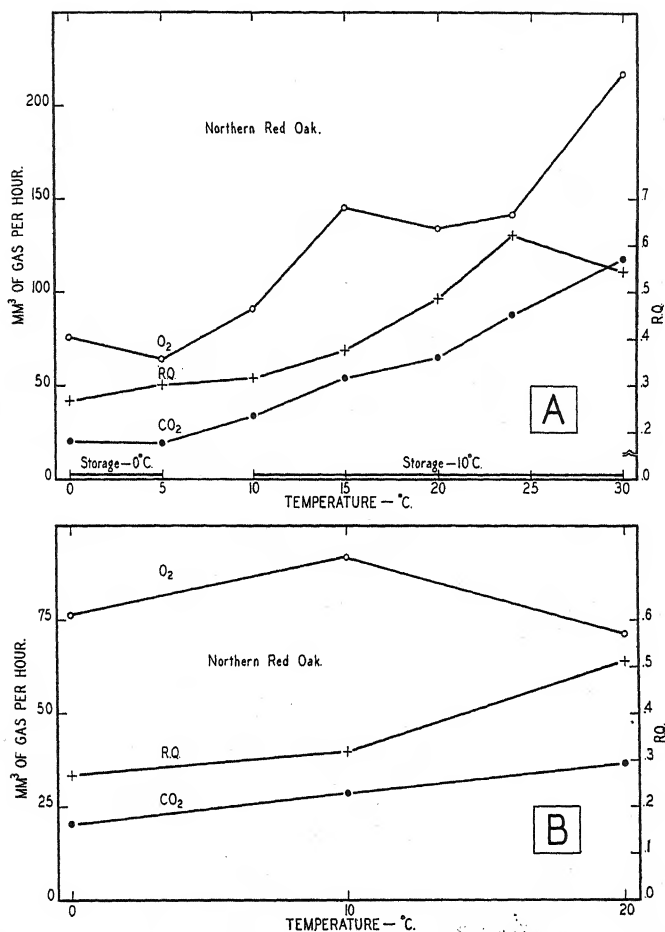


FIG. 4. A. The effect of different temperatures on the respiratory activity of northern red oak acorns after storage at 0° and 10° C. in 1936-1937.

B. The effect of storage temperature on the respiratory activity of northern red oak acorns in 1936-1937. Measurements were made at the storage temperatures.

#### EFFECT OF STORAGE TEMPERATURES ON R. Q. AND RATE OF RESPIRATION OF NORTHERN RED OAK ACORNS

The effect of storage temperatures on the R. Q. and rate of respiration of northern red oak acorns was investigated by measuring the O<sub>2</sub> consumption and CO<sub>2</sub> production of the acorns at the temperature at which they were stored (0°, 10°, and 20° C. in 1936-1937; 2.5°, 12.5°, 15.0° and 17.5° C. in 1937-1938).

Figure 4B shows a straight line relation between the rates of CO<sub>2</sub> production at 0°, 10°, and 20° C., increasing from the lowest storage tempera-



ture to the highest. The R. Q. increased gradually with temperature in the first stage, and more rapidly in the second stage. The O<sub>2</sub> consumption was practically the same at 0° and 20° but it reached its highest rate at 10° C. storage (table V).

The behavior of the northern red oak acorns the following year was somewhat different insofar as the O<sub>2</sub> consumption and CO<sub>2</sub> production were con-

TABLE III

EFFECT OF TEMPERATURE ON THE RESPIRATORY ACTIVITY OF NORTHERN RED OAK ACORNS

DATE	TEMPERATURE OF STORAGE	TEMPERATURE OF EXPERIMENT	GAS	NUMBER OF OBSERVATIONS	MEAN VOLUME OF GAS PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
Northern red oak								
	°C.	°C.			mm. <sup>3</sup>	mm. <sup>3</sup>	%	
1-15-37	0	0	CO <sub>2</sub>	31	20.50	1.69	8.23	0.2693
			O <sub>2</sub>	29	76.12	4.38	5.75	
1-16-37	0	5	CO <sub>2</sub>	27	19.48	2.54	13.03	0.3013
			O <sub>2</sub>	27	64.66	3.81	5.90	
1-17-37	10	10	CO <sub>2</sub>	30	29.10	2.52	8.66	0.3181
			O <sub>2</sub>	30	91.48	7.22	7.89	
1-18-37	10	15	CO <sub>2</sub>	30	54.52	3.74	6.85	0.3759
			O <sub>2</sub>	30	145.32	7.96	5.48	
2- 6-37	10	20	CO <sub>2</sub>	32	65.58	5.60	8.54	0.4888
			O <sub>2</sub>	32	134.16	6.34	4.72	
1-20-37	10	24	CO <sub>2</sub>	30	88.18	6.90	7.82	0.5208
			O <sub>2</sub>	30	142.04	10.42	7.34	
1-21-37	10	30	CO <sub>2</sub>	25	117.94	10.58	8.97	0.5442
			O <sub>2</sub>	25	216.72	17.71	8.17	

cerned but the R. Q.'s obtained at the different temperatures of the second year generally resemble the R. Q.'s obtained the first year.

Figures 6 and 7 have been constructed from the data presented in table VI. Figure 6 shows the respiratory activity of northern red oak acorns at different temperatures at various times during after-ripening while figure 7 shows the progress of respiratory activity at each storage temperature throughout the period of after-ripening. It is clearly shown in figures 6 and 7 that the R. Q. of northern red oak acorns decreases more rapidly during a period of storage at 2.5° C. than it does at the other temperatures.

#### CATALASE ACTIVITY

An experiment was performed to trace the catalase activity of both species through the storage period. Measurements were made at 25° C. of the

catalase activity of acorns from all of the storage lots at the time of storage and at the time of removal from storage. Another set of measurements was made of white oak acorns and northern red oak acorns which had been allowed a 24-hour germination period at 25° C. prior to preparing the material for measurement.

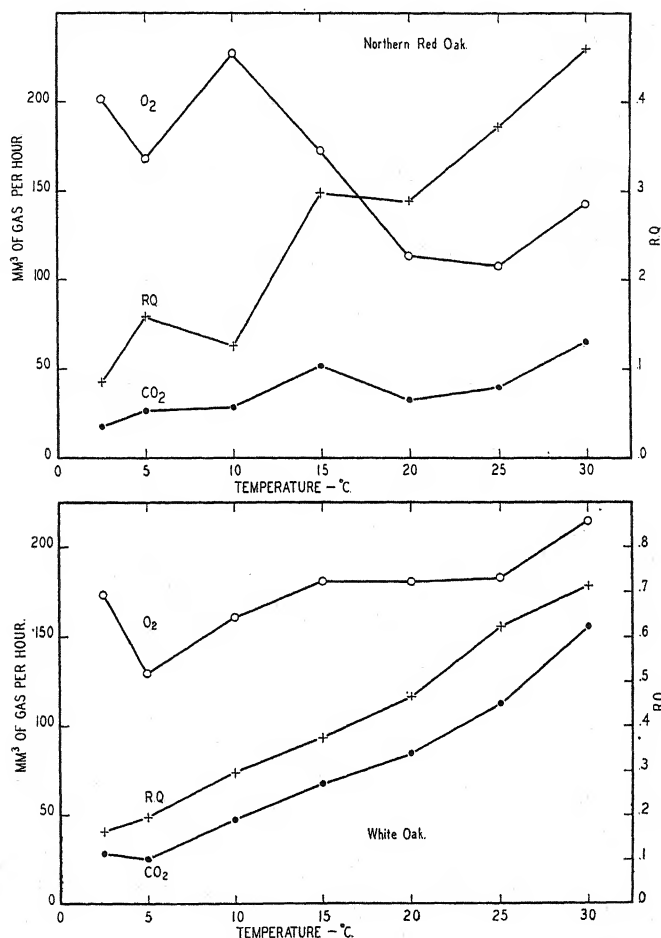


FIG. 5. The effect of different temperatures on the respiratory activity of northern red oak and white oak acorns after storage at 2.5° C. in 1937-1938.

The catalase activity of both species of acorns at 2.5° C. decreased slightly during the period of storage, although the activity of the white oak acorns was about three times that of the northern red oak acorns (figure 8A). The northern red oak acorns stored at 12.5°, 15.0°, and 17.5° C. showed an increase in activity during storage. The rate of catalase activity was lower in the lot stored at 15.0° than in the lots stored at 12.5° and 17.5° C.

The white oak acorns stored at 2.5° and allowed a 24-hour germination period at 25° C. showed an increase in catalase activity during the first half of the period of storage which was followed by a slight decrease in the latter

TABLE IV

NORTHERN RED OAK AND WHITE OAK ACORN RESPIRATION MEASURED AT 2.5°, 5°, 10°, 15°, 20°, 25°, AND 30° C. AFTER STORAGE AT 2.5° C.

DATE	TEMPER- ATURE OF EXPERI- MENT	GAS	NUMBER OF OBSER- VATIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRATORY QUOTIENT
Northern red oak							
	° C.			mm. <sup>2</sup>	mm. <sup>3</sup>	%	
1- 7-38	2.5	CO <sub>2</sub>	10	17.58	3.30	18.77	0.0871
		O <sub>2</sub>	10	201.74	14.22	7.05	
1- 7-38	5	CO <sub>2</sub>	10	26.64	3.64	13.66	0.1591
		O <sub>2</sub>	10	167.42	25.24	15.08	
1- 7-38	10	CO <sub>2</sub>	10	28.76	2.52	8.76	0.1270
		O <sub>2</sub>	10	226.44	20.50	9.05	
1- 8-38	15	CO <sub>2</sub>	10	51.52	19.40	37.66	0.2988
		O <sub>2</sub>	10	172.46	20.50	11.89	
1- 9-38	20	CO <sub>2</sub>	10	32.86	4.48	13.63	0.2891
		O <sub>2</sub>	10	113.66	9.60	8.45	
1- 9-38	25	CO <sub>2</sub>	10	39.96	8.14	20.37	0.3714
		O <sub>2</sub>	10	107.60	13.08	12.16	
1-10-38	30	CO <sub>2</sub>	10	65.64	15.70	23.92	0.4600
		O <sub>2</sub>	10	142.70	24.70	17.31	
White oak							
12-16-37	2.5	CO <sub>2</sub>	10	28.42	4.06	14.29	0.1636
		O <sub>2</sub>	10	173.68	9.72	5.60	
12-17-37	5	CO <sub>2</sub>	10	25.40	3.88	15.28	0.1952
		O <sub>2</sub>	10	130.14	10.94	8.41	
12-17-37	10	CO <sub>2</sub>	10	47.74	5.10	10.68	0.2977
		O <sub>2</sub>	10	160.34	6.10	3.80	
12-18-37	15	CO <sub>2</sub>	10	67.72	5.34	7.89	0.3737
		O <sub>2</sub>	10	181.22	16.92	9.34	
12-18-37	20	CO <sub>2</sub>	10	84.38	9.40	11.14	0.4673
		O <sub>2</sub>	10	180.56	15.32	8.48	
12-19-37	25	CO <sub>2</sub>	10	113.34	6.60	5.82	0.6211
		O <sub>2</sub>	10	182.48	9.34	5.12	
12-19-37	30	CO <sub>2</sub>	10	152.88	11.54	7.55	0.7127
		O <sub>2</sub>	10	214.52	10.20	4.75	

half. The northern red oak acorns subjected to the same temperature conditions showed a steady increase in catalase activity during the whole period of measurement (fig. 8B and table VII).

Lack of material prevented further catalase measurements at this time.

## GERMINATION TESTS

In 1936, 250 acorns of each species of oak were planted at the time of collection in a mixture of equal parts of sand and peat in a greenhouse to determine the percentage of germination. In 1937, 200 white acorns and 100 northern red oak acorns were planted in the same manner for the same purpose. The diurnal variation in temperature of the greenhouse was 55° to 90° F. (12.8° to 32.2° C.). The time of germination was arbitrarily chosen as the time when the plumule of the seedlings appeared at the surface of the ground.

The germination results indicate a great difference (figs. 9A and 10A) in temperature requirements for the germination of white oak acorns and the after-ripening and germination of northern red oak acorns. Within a period

TABLE V

EFFECT OF STORAGE TEMPERATURE ON THE RESPIRATORY ACTIVITY OF NORTHERN RED OAK ACORNS. RESPIRATORY MEASUREMENTS MADE AT STORAGE TEMPERATURES

DATE	STOR- AGE TEM- PERA- TURE	GAS	NUMBER OF OBSER- VATIONS	MEAN VOLUME OF GAS PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRA- TORY QUOTIENT
Northern red oak							
1-15-37	0	CO <sub>2</sub>	31	20.50	1.69	8.23	0.2693
		O <sub>2</sub>	29	76.12	4.38	5.75	
1-17-37	10	CO <sub>2</sub>	30	29.10	2.52	8.66	0.3181
		O <sub>2</sub>	30	91.48	7.22	7.89	
1-19-37	20	CO <sub>2</sub>	30	36.42	1.94	5.32	0.5101
		O <sub>2</sub>	30	71.40	3.22	4.50	

of 39 days after collection (fig. 9A) all of the viable white oak acorns had germinated. Of the 250 white oak acorns, 221 (88.4 per cent.) germinated. The following year (fig. 10A) 167 of 200 (83.5 per cent.) white oak acorns proved viable and had germinated in 35 days after collection. Both seasons some of the white oak acorns had protruding radicles at the time of collection showing the extent of their ability to germinate very soon after they fall from the tree.

Only one northern red oak seedling appeared (fig. 9A) in 40 days after planting and in 130 days 20 seedlings (8 per cent.) had appeared. At the time the experiment ended (July 10, 1937) 44 per cent. of the acorns had germinated. The following year (fig. 10A) 48 days elapsed before a seedling appeared and at the end of the experiment (February 16, 1938) 12 per cent. of the lot had germinated. It is apparent that the greenhouse temperatures were too high for the proper after-ripening of the northern red acorns.

Plots of 88 northern red oak acorns were set out in a greenhouse in 1936 to show the effect of three different storage temperatures ( $0^{\circ}$ ,  $10^{\circ}$ , and  $20^{\circ}$  C.) on the time of germination. The material had been kept in storage for

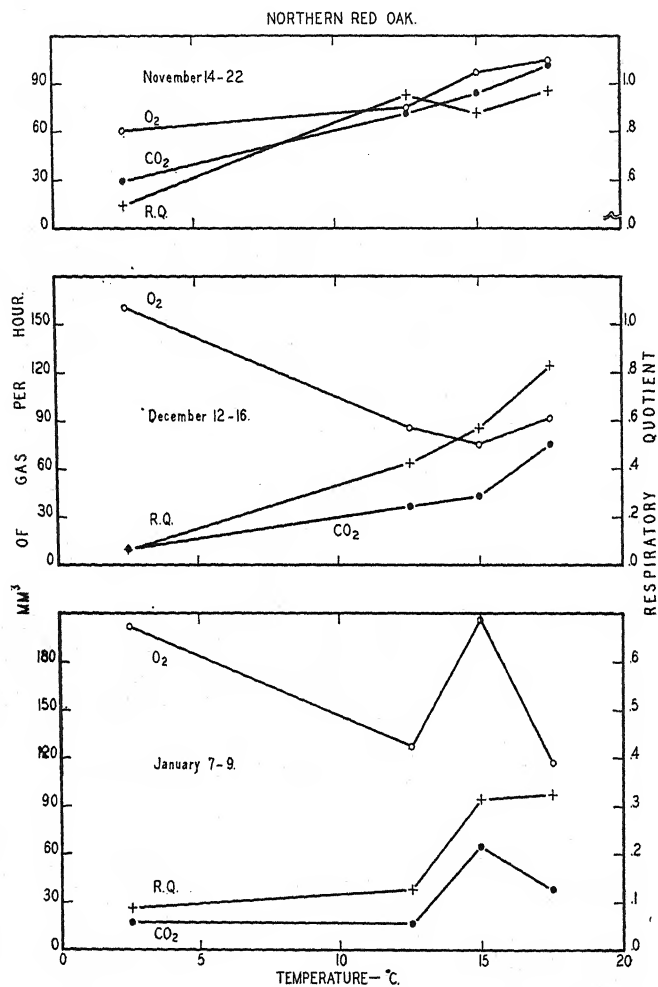


FIG. 6. Stages of respiratory activity of northern red oak acorns stored at different temperatures in 1937-1938. Measurements were made at the storage temperatures.

approximately two and one-half months. The following year (1937) 70, 79, 73, and 62 northern red oak acorns were set out under the same conditions except that they had been stored at  $2.5^{\circ}$ ,  $12.5^{\circ}$ ,  $15.0^{\circ}$ , and  $17.5^{\circ}$  C.

The 1936 germination tests on northern red oak acorns (fig. 9B) showed that in 30 days the material which had been stored at  $0^{\circ}$  C. had 83 per cent.,

TABLE VI

EFFECT OF STORAGE TEMPERATURES ON THE RESPIRATION OF NORTHERN RED OAK ACORNS  
DURING AFTER-RIPENING. MEASUREMENTS OF RESPIRATION WERE MADE  
AT THE STORAGE TEMPERATURES

DATE	GAS	NUM- BER OF OB- SERVA- TIONS	GAS PER GRAM DRY WEIGHT PER HOUR	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN	RESPIRA- TORY QUOTIENT
2.5° C.						
11-21-37	CO <sub>2</sub>	18	<i>mm.</i> <sup>3</sup> 29.94	<i>mm.</i> <sup>3</sup> 3.62	% 12.09	0.4990
	O <sub>2</sub>	18	60.00	5.20	8.67	
12-16-37	CO <sub>2</sub>	10	9.86	1.94	19.68	0.0613
	O <sub>2</sub>	10	160.88	11.98	7.45	
1- 7-38	CO <sub>2</sub>	10	17.58	3.30	18.77	0.0871
	O <sub>2</sub>	10	201.74	14.22	7.05	
12.5° C.						
11-22-37	CO <sub>2</sub>	18	71.14	5.78	8.12	0.9523
	O <sub>2</sub>	18	74.70	4.72	6.32	
12-16-37	CO <sub>2</sub>	10	36.78	4.58	12.45	0.4269
	O <sub>2</sub>	10	86.16	11.32	13.14	
1- 8-38	CO <sub>2</sub>	10	16.30	2.98	18.28	0.1272
	O <sub>2</sub>	10	128.16	9.68	7.55	
15° C.						
11-14-37	CO <sub>2</sub>	18	83.94	4.58	5.46	0.8677
	O <sub>2</sub>	18	96.74	5.46	5.64	
12-12-37	CO <sub>2</sub>	10	43.18	3.82	8.85	0.5724
	O <sub>2</sub>	10	75.44	6.10	8.09	
1- 8-38	CO <sub>2</sub>	10	64.98	16.00	24.62	0.3153
	O <sub>2</sub>	10	206.10	22.36	10.85	
17.5° C.						
11-15-37	CO <sub>2</sub>	18	101.48	8.84	8.71	0.9711
	O <sub>2</sub>	18	104.50	8.52	8.15	
12-12-37	CO <sub>2</sub>	10	75.88	7.90	10.41	0.8293
	O <sub>2</sub>	10	91.50	9.54	10.43	
1- 9-38	CO <sub>2</sub>	10	38.12	8.02	21.04	0.3254
	O <sub>2</sub>	10	117.18	9.94	8.48	

that stored at 10° C. 93 per cent., and that stored at 20° C. only 6.8 per cent. germination. The following year the total percentages of germination were 68.6, 83.5, 69.9, and 43.6 for material which had been stored at 2.5°, 12.5°, 15.0°, and 17.5° C., respectively (fig. 10B). Both years the lowest storage temperatures were not favorable for the highest percentage of total ger-

mination nor did germination start first in these lots when placed under conditions favorable for germination. Northern red oak acorns stored at 10° and 12.5° C. started germinating sooner, germinated more rapidly and had a higher percentage of germination than acorns of the same species stored at other temperatures in these experiments.

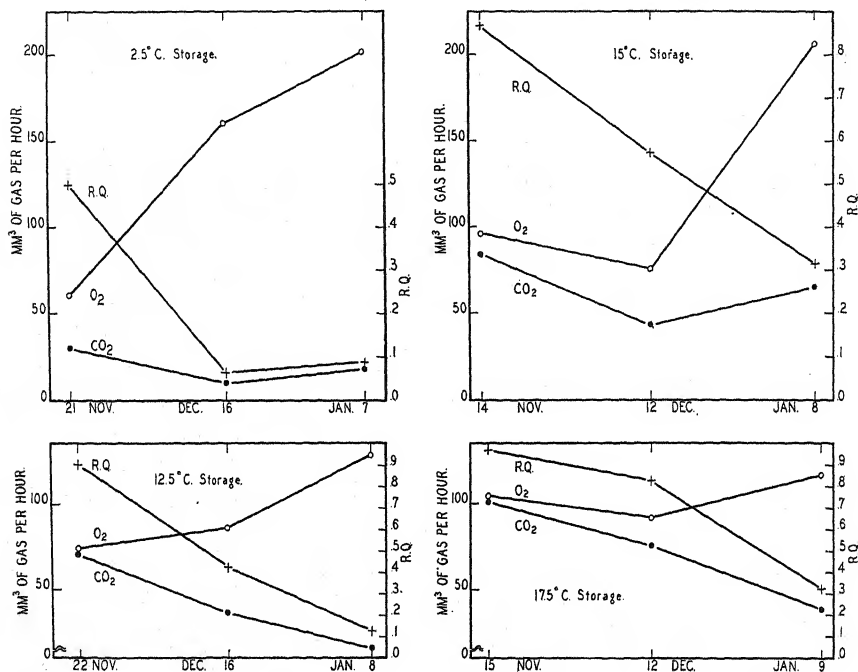


FIG. 7. The effect of storage temperatures on the respiratory activity of northern red oak acorns in 1937-1938. Measurements were made at the storage temperatures.

## Discussion

### TIME REQUIRED TO ADJUST ACORNS TO A NEW TEMPERATURE

The data presented in figure 1 indicate that at least 6 hours were required for the rate of respiration to become constant when the temperature of the acorns was increased from 10° to 20° C. The time required would probably have been longer if the fruit coats had not been removed. Evidently, overnight storage in a CO<sub>2</sub>-free atmosphere at the experimental temperature allowed sufficient time for the establishment of a new equilibrium with respect to CO<sub>2</sub> exchange between the acorns and their environment. It is probable that any marked change in the environment of living material, such as removal of seed or fruit coats, changes in temperature, or in the concentration of the surrounding atmosphere, should be followed by a sufficient

time interval to allow establishment of physical and physiological equilibrium under the new conditions. The time interval required for any particular material can probably be determined only by experiment.

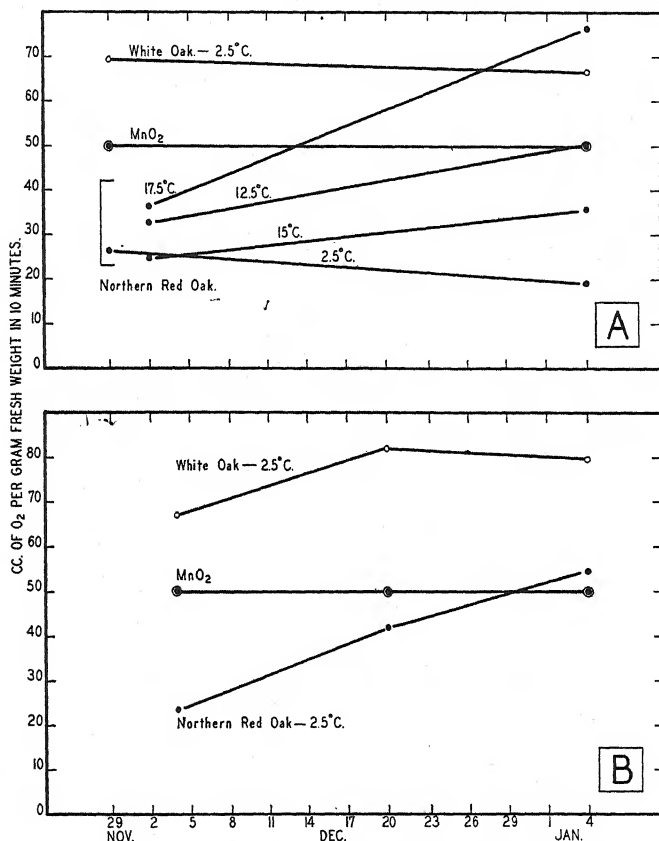


FIG. 8. A. The changes in catalase activity of northern red oak acorns during storage at different temperatures in 1937-1938. Measurements were made at 25° C. immediately after removal from storage.

B. The changes in catalase activity of white oak and northern red oak acorns during storage at 2.5° C. in 1937-1938. Measurements were made at 25° C. after a 24-hour germination period at that temperature.

#### COMPARISON OF RESPIRATION IN TWO SPECIES OF OAK ACORNS

The production of CO<sub>2</sub> by acorns of the two species when stored and measured at 0° and 2.5° C. was consistently low during the entire storage period, that of the northern red oak being the lower. During the storage period the O<sub>2</sub> consumption of the white oak acorns increased to a very high level which was maintained during the latter part of the period. The O<sub>2</sub>



consumption of the northern red oak acorns was lower than that of the white oak acorns at the beginning of the season. It increased during the period of storage and in 1937-1938 reached a higher level during midwinter than was reached by white oak acorns stored at the same temperature.

TABLE VII

EFFECT OF STORAGE TEMPERATURE AND GERMINATING CONDITIONS ON THE CATALASE ACTIVITY OF WHITE OAK AND NORTHERN RED OAK ACORNS AT 25° C. (ADJUSTED TO  $\text{MnO}_2$  CONTROL). WO = WHITE OAK; NRO = NORTHERN RED OAK

DATE	STORAGE TEMPERATURE	MATERIAL	NUMBER OF OBSERVATIONS	O <sub>2</sub> PRODUCED IN 10 MINUTES	STANDARD ERROR OF MEAN	STANDARD ERROR OF MEAN
Without germination period						
	°C.			ml.	ml.	%
11-29-37	2.5	WO	9	69.33	2.03	2.92
	2.5	NRO	9	26.40	0.97	3.69
		$\text{MnO}_2$	4	50.00	0.54	1.08
12- 2-37	12.5	NRO	5	32.70	1.20	3.67
	15.0	NRO	5	24.50	1.03	4.18
	17.5	NRO	5	36.33	1.50	4.20
1- 4-38	2.5	WO	5	66.45	0.95	1.43
	2.5	NRO	5	19.15	0.88	4.57
	12.5	NRO	5	50.26	2.23	4.23
	15.0	NRO	5	35.90	1.13	3.13
	17.5	NRO	5	76.15	1.35	1.77
		$\text{MnO}_2$	3	50.00	0.85	0.68
With germination period						
12- 4-37	2.5	WO	5	66.90	1.03	1.53
	2.5	NRO	5	23.50	1.25	5.32
		$\text{MnO}_2$	4	50.00	0.54	1.08
12-20-37	2.5	WO	5	82.00	1.95	2.38
	2.5	NRO	5	42.08	1.78	4.22
		$\text{MnO}_2$	4	50.00	1.44	2.88
1- 4-38	2.5	WO	5	79.73	1.83	2.29
	2.5	NRO	5	54.30	0.88	1.61
		$\text{MnO}_2$	3	50.00	0.85	0.68

The respiratory quotients (R. Q.'s) are somewhat similar in the two species both being very low and both showing a decrease during the storage period. A difference in R. Q.'s might have been expected since KORSTIAN (6) found northern red oak acorns to contain a much higher percentage of fats than white oak acorns. The actual decreases in fat content were rather similar, however, in the two species. Apparently the same sort of metabolic activities occur in acorns of both species. The fact that the  $\text{CO}_2$  production was relatively constant during the entire storage period suggests that the

rate of cellular respiration may have been approximately constant. The increased  $O_2$  consumption may have resulted from an increase in rate of conversion of fats into carbohydrates, a process which requires large quantities of oxygen and which may even result in an increase in dry weight.

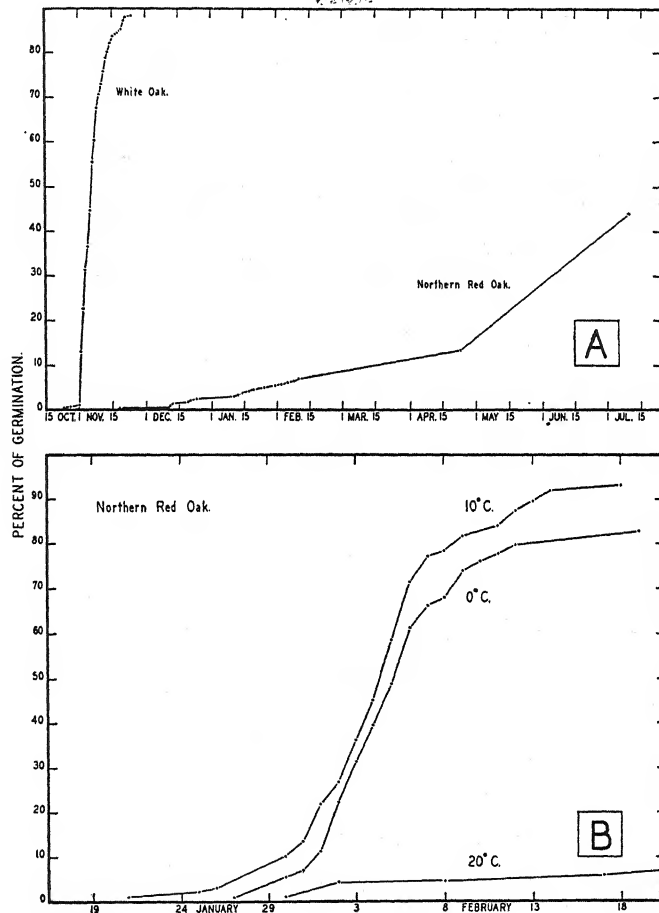


FIG. 9. A. The difference in germination of white oak and northern red oak acorns which have been planted in a greenhouse at the time of collection in 1936-1937.

B. The effect of storage temperatures on the germination of northern red oak acorns in 1936-1937.

KORSTIAN (6) reported that during the storage period from November to April the fat content of both red and white oak acorns decreased while the carbohydrate content increased. The low R. Q.'s found in the present experiments are such as would be expected if fats are being converted to carbohydrates rather than being completely oxidized. MILLER (9) in his

summary of the literature dealing with chemical changes in germination of oily seeds mentions a number of instances where the fat content decreased while the carbohydrate content increased.

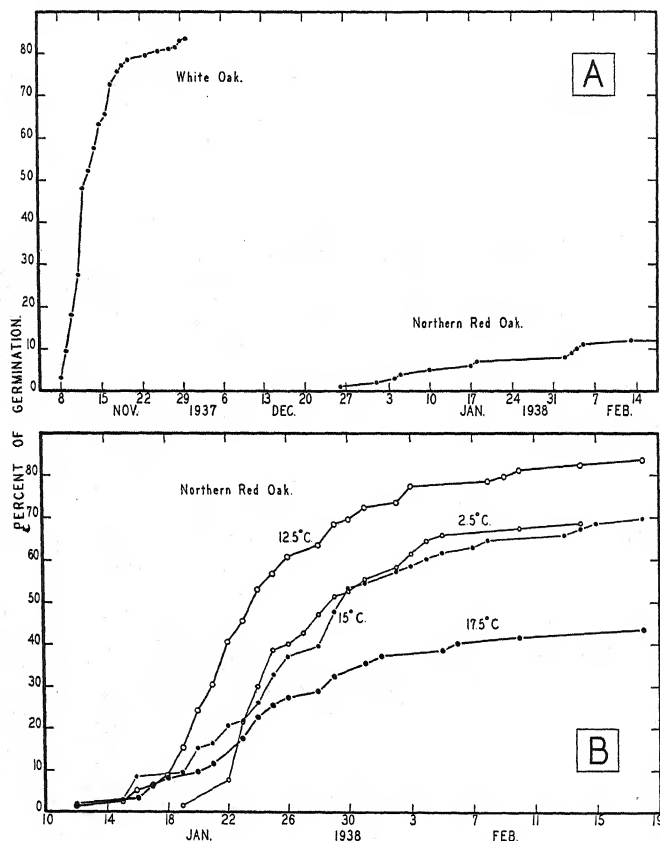


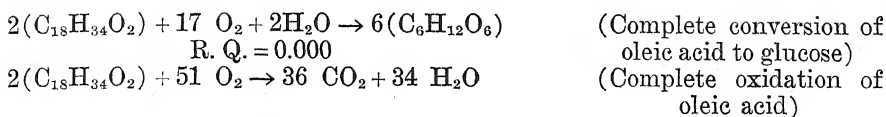
FIG. 10. A. The difference in germination of white oak and northern red oak acorns which have been planted in a greenhouse at the time of collection in 1937-1938.

B. The effect of storage temperatures on the germination of northern red oak acorns in 1937-1938.

HUTCHINS (5) found that oil from acorns of *Quercus palustris* had the following composition:

	PERCENTAGE
Glycerides of linoleic acid .....	27.92
Glycerides of oleic acid .....	57.01
Glycerides of saturated acids .....	15.01

The following formulae show how any oleic acid present in acorns may be oxidized:



$$\text{R. Q.} = \frac{36}{51} = 0.706$$

These theoretical equations show that the R. Q. of oleic acid may extend over a range of 0.000 to 0.706 depending on the proportion converted to stored carbohydrates and the proportion oxidized following digestion. The behavior of oleic acid is representative of what might be expected of other fatty acids present in acorns.

There is a possible indication, shown by the constant  $\text{CO}_2$  production (fig. 3), that the oxidation of the carbohydrates proceeded at a constant rate throughout the whole period of storage but that the conversion of fatty acids to carbohydrates increased rapidly between November 21 and December 16, 1937, where it reached an equilibrium which existed until the end of the storage period.

#### EFFECTS OF TEMPERATURE ON RESPIRATION OF ACORNS

The result of studies of the rates of respiration at different temperatures are not entirely consistent, especially for northern red oak. Both species showed a consistent increase in  $\text{CO}_2$  production and in R. Q. with increasing temperature both years (figs. 4A and 5). The  $\text{O}_2$  consumption of white oak acorns did not change materially with a change in temperature. The  $\text{O}_2$  consumption of northern red oak acorns increased with temperature in 1936-1937, but in 1937-1938 it decreased. No explanation of this variation can be offered.

The consistently increasing R. Q.'s of both species indicate that possibly as the temperature increases there is a change from carbohydrate accumulation, as a result of conversion of fatty acids in excess of oxidation of carbohydrates, to a greater rate of oxidation of carbohydrates.

#### EFFECT OF STORAGE TEMPERATURES ON R. Q. AND RATE OF RESPIRATION OF NORTHERN RED OAK ACORNS

It is found that the lower storage temperatures are accompanied by lower R.Q.'s and higher percentages of germination when planted at greenhouse temperatures (figs. 4B, 7, 9B, and 10). Figure 7 is possibly indicative of a more efficient accumulation of carbohydrates at the  $2.5^\circ$  and  $12.5^\circ$  C. storage temperatures. It is possible that the material stored at  $2.5^\circ$  C. reached an efficient stage of carbohydrate accumulation earlier and was apparently subjected to it for a longer period than the other 3 lots stored at different temperatures. The germination results of this lot were almost the same as the lot which was stored at  $15^\circ$  C. The lot stored at  $12.5^\circ$  reached this efficient

stage of accumulation at the end of the storage period and then had the highest percentage and quickest germination of the 4 lots. The lots of material which were stored at 15° and 17.5° C. did not reach an R.Q. lower than 0.310 while the R.Q.'s of the lots stored at 2.5° and 12.5° C. were as low as 0.061 and 0.127 (table VI). It seems possible that the accumulation of carbohydrates varies inversely with the storage temperature in these experiments but that the best physiological balance has been obtained by storage of the acorns at 10° or 12.5° C. There was a noticeable lag in the germination of the lot stored at 2.5° C. (fig. 10B) which may have been caused by a secondary dormancy. Further investigation of this point is desirable.

Apparently, northern red oak acorns must be subjected to conditions allowing a period of carbohydrate accumulation before they become properly after-ripened. The successful use of Warburg respirometers for an indication of the stage of after-ripening will depend on the length of period necessary for after-ripening, which in turn will depend on the temperature at which the material is stored. From the present study there is an indication that after-ripening is completed in 6 weeks of storage at 12.5° C. while there is a possibility that 3 weeks of storage at 2.5° C. is sufficient for this process (fig. 7). This point could be confirmed by removing the acorns from low temperature storage (2.5° C.) to conditions favorable for germination as soon as the R.Q. of the material has reached a low level (0.1 to 0.2). It has been pointed out by KORSTIAN (6) that after-ripening was hastened by temperatures of 50° to 65° F. but while these temperatures may furnish conditions for optimum germination it is possible that lower temperatures are more favorable for quicker after-ripening.

Undoubtedly, the best measurements of respiration are those which include the consumption or uptake of O<sub>2</sub> and the production of CO<sub>2</sub>. There are methods of measuring respiration however, which are concerned with the measurement of the exchange of only one of these gases. It has been found in this study that the production of CO<sub>2</sub> is more constant than the consumption of O<sub>2</sub> and, therefore, is less affected by chemical transformations of food in the respiring material. Where both after-ripening and respiration are concerned, a measurement of the CO<sub>2</sub> output does not offer as accurate an indication of any chemical changes of food as a measurement of the O<sub>2</sub> consumption.

#### CATALASE ACTIVITY

BALDWIN (3) suggested the following catalase test of seed viability as having been found applicable to several coniferous species:

When the quotient 
$$\frac{\text{O}_2 \text{ released from H}_2\text{O}_2 \text{ by catalase of stimulated seed}}{\text{O}_2 \text{ released from H}_2\text{O}_2 \text{ by catalase of resting seed}}$$
 is greater than unity the seeds are considered viable.

In the present study such an indication of viability was found to be of little value for northern red oak acorns, since catalase activity is not directly related to after-ripening and the latter seems to be the controlling factor affecting germination. Viable northern red oak acorns will not germinate unless they are properly after-ripened. Tests based on catalase activity indicated that neither white oak nor northern red oak acorns were viable at the beginning of the experiment, yet later by the same test they were viable.

Catalase activity does not offer a measure of after-ripening (fig. 8A) since the lot of acorns stored at 17.5° C. shows the greatest catalase activity at the end of the storage period and yet has the lowest percentage of germination apparently because of incomplete or insufficient after-ripening. AFANASIEV (1) also found that catalase activity was apparently independent of the degree of after-ripening.

#### GERMINATION

AIKMAN (2) found that the germination of northern red oak acorns was markedly slower than the germination of white oak, black oak, and bur oak although 94 per cent. germination was obtained after 138 days. This percentage was obtained on a basis of 20 acorns of each species planted directly in a greenhouse where the temperature varied from 55° to 90° F. The results obtained in the present study do not agree with the results obtained by AIKMAN. In 1936-1937, 250 northern red oak acorns planted directly in a greenhouse having approximately the same temperature range as that used by AIKMAN gave 8 per cent. germination; and 100 acorns of the same species planted the following year gave 12 per cent. germination, in about the same time interval used by AIKMAN.

KORSTIAN (6) found that an average night temperature of 50° and 65° F. during the day gave the most satisfactory results with red, black, white, scarlet, and chestnut oak acorns when both promptness of germination and total germination were considered. Records of diurnal variations of greenhouse temperatures were not kept in the present study.

#### SUGGESTIONS FOR FURTHER STUDY

The measurement of respiration during the period of storage of white oak and some acorns of the red or black oak group stored at 0°, 2.5°, 5°, 7.5°, 10°, 12.5°, and 15° C., supplemented with germination tests, should definitely indicate the best storage temperature for the rate of after-ripening desired. At the time when the lots show an R.Q. near 0.3, germination plots should be set out weekly or bi-weekly thereafter until the R.Q. remains constant for at least two weeks. The results of these germination tests will establish the limits of use of the R.Q. as an indicator of after-ripening.

#### Summary

Measurements of respiration, catalase activity and germination of white oak (*Quercus alba* L.) and northern red oak [*Quercus borealis* var. *maxima*

(Marsh.) Ashe] acorns which had been stored at 0° and 2.5° C. were made in order to obtain an indication of the physiological differences between the two species of acorns during the over-wintering period. Measurements of respiration and germination tests of northern red oak acorns which had been stored at 0°, 2.5°, 10°, 12.5°, 15°, 17.5°, and 20° C. were made during the overwintering period to determine the physiological changes and the progress of after-ripening of the acorns at these different temperatures.

1. Northern red oak acorns approach constant respiratory activity after 6 hours of exposure to a new constant temperature.

2. Although the rates of gas exchange may differ slightly the physiological activity of both species of acorns is very similar as indicated by similar respiratory quotients when the acorns are stored at 0° and 2.5° C.

3. The CO<sub>2</sub> production of both species of acorns remained constant during overwinter storage at 2.5° C. while the O<sub>2</sub> consumption of white oak acorns increased markedly after the first 3 weeks of storage and the O<sub>2</sub> consumption of northern red oak acorns increased rapidly during the first 3 weeks of storage. Because of this behavior the respiratory quotients were lowered from about 0.50 to 0.09 and 0.16 respectively where they remained constant during the last 3 weeks of storage, possibly indicating a period of carbohydrate accumulation.

4. The respiratory quotients of both species of acorns generally increased with temperature when removed from storage at 2.5° C., suggesting a greater use of carbohydrates at the higher temperatures.

5. The respiratory quotient of northern red oak acorns stored at temperatures of 2.5°, 12.5°, 15°, and 17.5° C. decreased more rapidly at the lowest storage temperature. This behavior suggests that possibly carbohydrate accumulation started earlier and proceeded at a greater rate at the lower storage temperatures.

6. Measurements of catalase activity did not materially aid in interpreting the progress of after-ripening or the potential germinating ability of the acorns.

7. Germination tests in the greenhouse of northern red oak acorns which had been previously stored at 0°, 2.5°, 10°, 12.5°, 15°, 17.5°, and 20° C. for two months showed that storage temperatures of 10° and 12.5° C. were best for the germination of northern red oak acorns.

8. The northern red oak acorns which showed the highest percentage of germination had a respiratory quotient lower than 0.3 in storage.

The writer expresses his gratitude for the helpful criticism of Dr. PAUL J. KRAMER during the course of this study and for his material assistance in the preparation of this paper. Indebtedness is acknowledged to Professor FRANCIS X. SCHUMACHER for suggestions on the sampling procedure and the

statistical treatment of the data, and to Dr. CLARENCE F. KORSTIAN for advice on the treatment of acorns for storage.

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# DISTRIBUTION OF NITROGENOUS FRACTIONS, SUGARS, AND OTHER SUBSTANCES IN ANANAS GROWN IN DARKNESS VERSUS DAYLIGHT<sup>1</sup>

C. P. SIDERIS, B. H. KRAUSS, AND H. Y. YOUNG

(WITH FOURTEEN FIGURES)

## Introduction

The object of the present studies is to report and compare data on the nitrogen metabolism of two sets of plants, both of which were in an early stage of fruiting at the time of shifting some of them to continual darkness, and leaving the remainder under the seasonal light conditions of a glass house. The former set was kept in a darkened box for four months during which time there was little expansion of new leaf tissue since, in the pineapple, leaf growth practically ceases after sexual differentiation. The small fruits already formed at the start of the experiment increased in size and ripened. Since the period of darkness was quite long the analytical results obtained were decidedly different for the two sets of plants.

The literature on nitrogen metabolism has been reviewed in the first paper (6) of this series. Since that time a paper by VICKERY *et al.* (9) has appeared which contrasts the physiological behavior of excised matured tobacco leaves in light and darkness. MURNEEK, in a recent paper (3), contrasted the relationships between carbohydrates and nitrogen in connection with photoperiodism. BURKHOLDER (1) has reviewed very extensively the rôle of light in the life of plants. The reviews on nitrogen metabolism by NIGHTINGALE (4) and MCKEE (2) practically bring our knowledge of experimental work on the physiological rôle of nitrogen in plant nutrition up to date.

## Experimental methods

Two sister shoots of uniform weight and appearance were removed from each of several mother plants which were growing in the field. Each of the shoots were at the red bud stage; that is, when the fruit bud was visible between the apical leaves of the stem but had not yet emerged completely. One of each set of the shoots was placed in a group grown under seasonal greenhouse conditions and the other in a group grown under conditions of continual darkness. The shoots in both groups however, were grown for the first month in the greenhouse under identical quantities and qualities of light to allow them to produce good root systems and new leaves. At the end of this period, *i.e.*, on February 15, 1935, the plants of one of the groups were transferred to a box, ten feet long, four feet wide, and four feet high; venti-

<sup>1</sup> Published with the approval of the Director as Technical Paper no. 114 of the Pineapple Experiment Station, University of Hawaii.

lated with two fans, one drawing air inwards and the other outwards, and kept dark at all times except during the renewal of nutrient solutions.

The nitrate-complete nutrient solution was changed weekly, at which time the volumes of absorbed and evaporated water were determined. Livingston atmometers were set up in the darkened box and in the greenhouse to obtain records of the rate of water evaporation under the two environments. Constant temperature records obtained in both environments fluctuated between 70° and 85° F. The temperature in the greenhouse was about 5 degrees higher between the hours of 11 A.M. and 4 P.M. than in the box. At all other times the temperature values were approximately the same.

The plants were kept in their respective environments until the fruits matured. The fruits of the plants kept in the darkened box matured and ripened about one month earlier than those in the greenhouse. Consequently, the former plants were harvested on June 15, 1935, *i.e.*, after four months of continuous darkness. The plants kept in the greenhouse and exposed to light were harvested on July 15, 1935. The whole plant was harvested and its different organs were cut into as many sections as were deemed necessary for the proper interpretation of the physiological rôle of different plant organs and tissues in the metabolism of the plants under the conditions of the two experimental environments. The different plant sections were made in accordance with our previously described plan (7) which is shown for convenience in figure 1. They were prepared and analyzed with the technique and methods described in former publications (6, 7, 8).

The leaves, after detachment from the stem, were segregated into two lots. Lot I comprised the thoroughly mature and active leaves of groups C and D which had probably attained their maximum growth at the beginning of the experiment. Lot II comprised the younger leaves of the plant, *i.e.*, those belonging to groups E and F of fruit-bearing plants, as indicated in a former paper (7). The latter leaves had not completed growth at the beginning of the experiment but, at the time of harvest, they were thoroughly developed and mature. The terminal chlorophyllous sections, 4 and 5, of the leaves of groups (C and D, E and F) of the plants kept in darkness showed symptoms of chlorophyll breakdown and tissue dehydration and were considered dead at harvest. The latter were, of course, analyzed as separate plant fractions. The stem was separated into a basal section, comprising the region on which the bases of the leaves of groups B and C were attached, and into an apical or upper section to which the bases of the leaves of D and E groups were attached. The peduncle was separated from the fruit and stem.

At harvest time the crown, or apical vegetative outgrowth, was detached from the fruit and the latter dissected, separating the flesh from the shell tissues as in figure 1. The stem and leaf tissues of the crown were analyzed as a single unit.

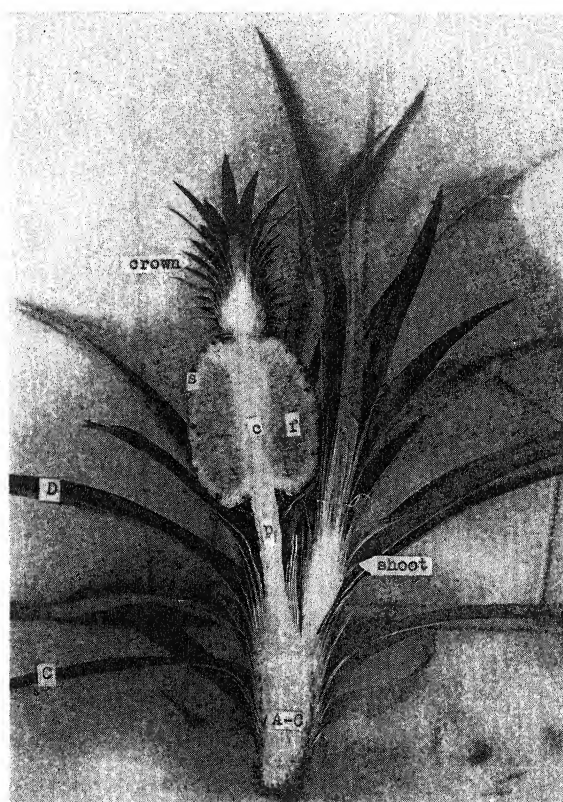


FIG. 1. Longitudinal section of a pineapple plant at fruiting stage showing the stem and its two sections (A-C) and (D-E); peduncle (p), the flesh (f), core (c), shell (s), and crown of the fruit; the (B), (C), (D), and (E) groups of the leaves, and the shoot.

## Results

### PLANT AND FRUIT WEIGHTS

Corresponding plants in the two lots were sisters and of about the same weight at the beginning of the experiment. The plants grown in darkness did not gain as much in weight as those grown in light, as will be seen by observation of figure 2. In fact, the former plants actually lost weight as a result of the death of the terminal portions of the older leaves. The fruits of the lot of plants grown in light weighed about twice as much as those of the lot grown in darkness. The crowns of the fruits of the former series weighed four times as much as those of the latter. The longer growth period and exposure to light were probably responsible for the greater amounts of plant and fruit tissue produced by the plants grown in the greenhouse.

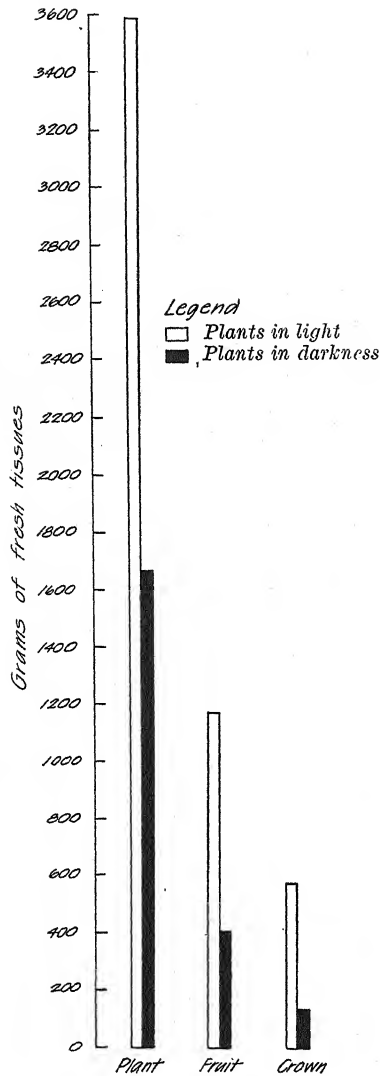


FIG. 2. Grams of fresh weight of plants, fruits, and crowns produced by two sets of pineapple sister shoots; one grown for 5 months in light, and the other for 4 months in darkness.

#### WATER ABSORPTION AND EVAPORATION

The volume of water absorbed by the two groups of plants was different, being greater for the plants exposed to light and less for those kept in darkness, as shown in figure 3. The relative degree of water evaporation, as determined with calibrated LIVINGSTON atmometers, varied slightly under

the two different environments, as shown in figure 4. In figure 5, where the data on water absorption are presented on a percentage basis, it will be seen that the plants exposed to sunlight absorbed approximately three to four times more water than those kept in darkness. The amounts of water evaporated by atmometers, however, under the two different environments were

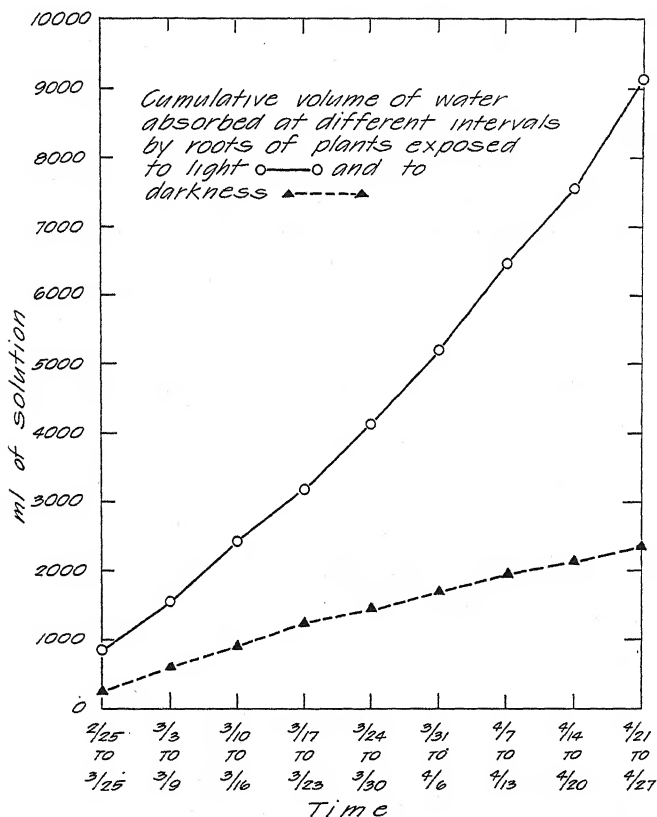


FIG. 3. Milliliters of nutrient solution absorbed by two sets of pineapple sister shoots; one grown in greenhouse light, and the other in darkness.

only slightly different, as the atmometer in the greenhouse evaporated only about 1.2 times more water than the one in the darkened box. The greater amounts of water absorbed by the plants in the greenhouse may be accredited in part therefore, to conditions more favorable for photosynthesis and transpiration. Moreover, the plants grown in the greenhouse had more and better roots than those in the darkened box. This was not unexpected with the greater production of carbohydrate materials which in many cases have been found to be essential for root formation and functioning. The smaller

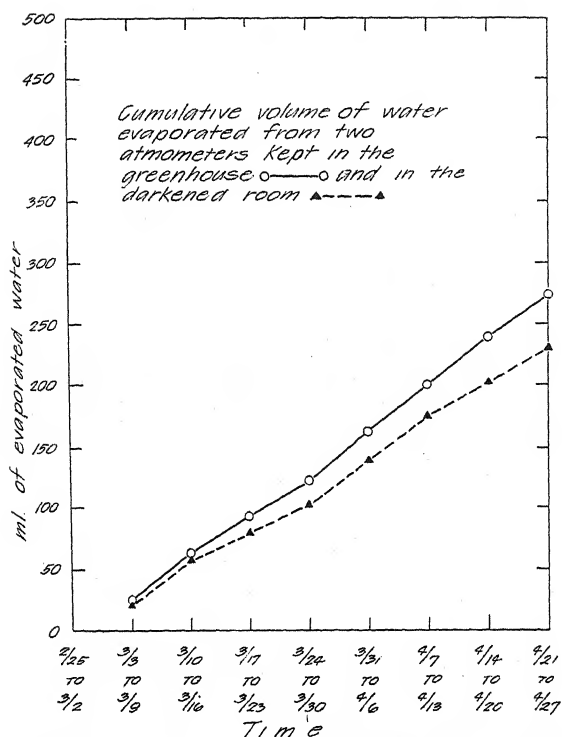


FIG. 4. Milliliters of water evaporated by LIVINGSTON atmometers; one kept in the greenhouse, and the other in the darkened box.

amounts of water absorbed by the plants in the darkened box were chiefly due to decreased growth.

#### TOTAL SOLIDS OF FRUITS

The distribution of total solids, determined refractometrically, is reported in figure 6 which shows that the percentage of total solids was greater in the fruit tissues of the plants exposed to light than in the tissues of those kept in darkness. In both groups, the tissues of the flesh contained slightly greater amounts of solids than those of the shell. This difference may not be of any physiological significance, because the tissues of the flesh accumulate, as a general rule, greater quantities of such solids as sugars than do those of the shell.

#### ACIDITY OF FRUITS

The fruits produced by both lots of plants varied considerably in acidity. The acid of the sap was titrated against 0.1 N NaOH and is reported as per cent. citric acid. The amounts of titratable acidity as citric acid in the various sections of the fruits are presented in table I and figure 7.

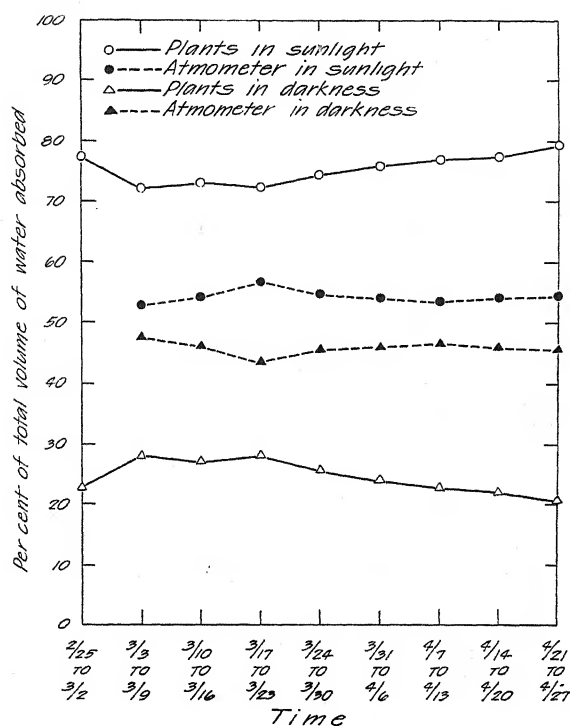


Fig. 5. Percentage of the total volume of water absorbed by the two sets of plants and also of that evaporated by the atmometers under the two different environmental conditions.

TABLE I

INITIAL AND FINAL PLANT WEIGHTS, FRUIT WEIGHTS, TITRATABLE ACIDITY, AND TOTAL SOLUBLE SOLIDS CONTENT OF TWO LOTS OF PLANTS, ONE EXPOSED TO LIGHT AND THE OTHER KEPT IN DARKNESS FROM AN EARLY STAGE OF FRUIT DEVELOPMENT

ITEMS	SERIES OF PLANTS	
	IN LIGHT	IN DARKNESS
Experiment started .....	2/15/35	2/15/35
Experiment harvested .....	7/13/35	6/17/35
Initial total weight, gm. ....	1900	1900
Final total weight, gm. ....	3585	1657
Final fruit weight, gm. ....	1175	406
Final crown weight, gm. ....	565	126
Titratable acidity* of shell base .....	1.15%	0.38%
Titratable acidity of shell apex .....	1.20%	0.34%
Titratable acidity of flesh base .....	0.90%	0.75%
Titratable acidity of flesh apex .....	0.95%	0.64%
Total soluble solids† of shell base .....	10.40%	3.20%
Total soluble solids of shell apex .....	9.80%	3.20%
Total soluble solids of flesh base .....	12.20%	3.80%
Total soluble solids of flesh apex .....	11.00%	4.00%

\* Reported as per cent. citric acid.

† Determined refractometrically.



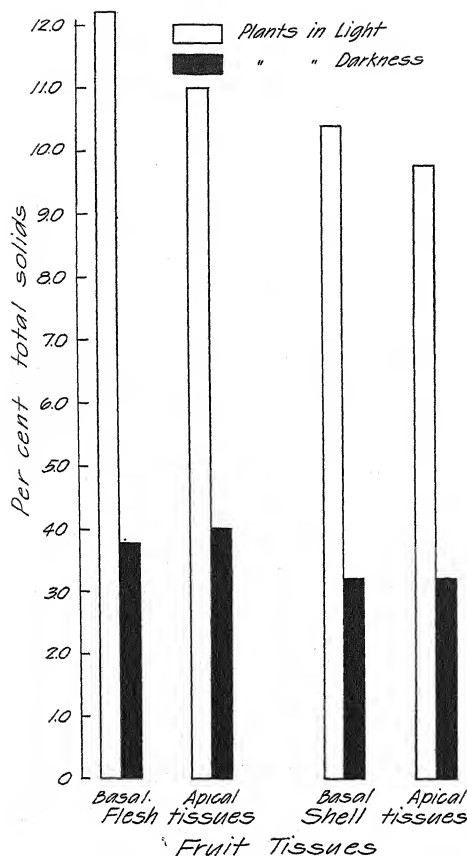


FIG. 6. Percentage of total solids, determined refractometrically, of the basal and apical tissues of the flesh and shell of fruits of pineapple sister shoots either exposed to greenhouse light or kept in darkness.

The data show that total acids in the flesh and shell tissues of the fruits of the plants exposed to light were considerably greater than those of corresponding sections of the fruits of the plants grown in darkness.

#### DISTRIBUTION OF NITROGENOUS FRACTIONS

The data for the distribution of different fractions of nitrogen in the various sections of the leaves of groups C-D and E-F, and of the stem and fruit of plants exposed either to light or kept in darkness are presented in tables II and III and in figures 9 to 11.

Reasons for the segregation of leaves and stem of pineapple plants into groups of different chronological and physiological ages, and for their sectioning into homogeneous lots, were presented and amply discussed in a former publication (7).

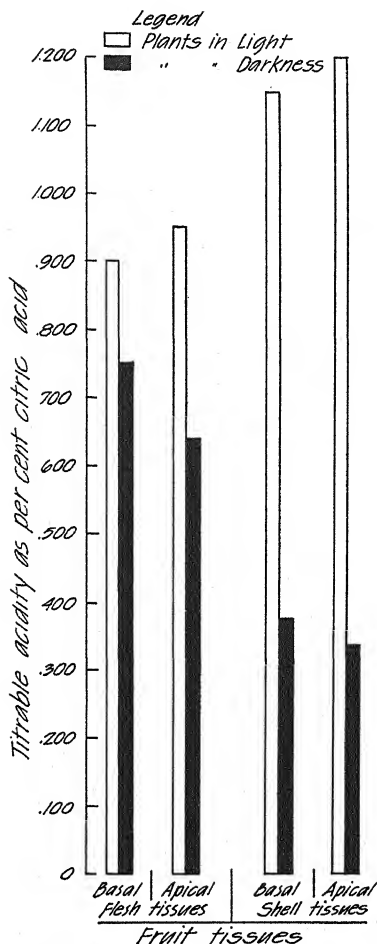


FIG. 7. Titratable acidity, as per cent. citric acid, of the basal and apical tissues of the flesh and shell of fruits of pineapple sister shoots either exposed to greenhouse light or kept in darkness.

The data for the mature leaf groups C and D of the plants kept in light and in darkness, as presented in figures 8-A and 8-B, show the following conditions:

1. Ammonium was found in very small quantities, while nitrate was present in considerably greater amounts in the basal non-chlorophyllous sections.
2. The quantities of glutamine were generally small. Those of asparagine were many times greater.
3. The quantities of mono-amino and basic nitrogen fractions were great in both the plants kept in light and in darkness. The quantities in the plants

TABLE II

DISTRIBUTION OF DIFFERENT FRACTIONS OF INORGANIC, SOLUBLE ORGANIC, AND INSOLUBLE ORGANIC NITROGEN IN THE TISSUES OF THE LEAVES, STEM, FRUIT, AND ROOTS OF *Ananas comosus* (L.) MERR. GROWN IN NITRATE SOLUTION CULTURES AND UNDER SEASONAL GREENHOUSE LIGHT CONDITIONS FOR FIVE MONTHS

PLANT SECTIONS	MILLIGRAMS PER GRAM OF FRESH TISSUE									
	INORGANIC N		SOLUBLE ORGANIC NITROGEN				INSOLUBLE ORGANIC NITROGEN			
	AMMONIUM	NITRATE	GLUTAMINE	ASPARAGINE	MONO-AMINO	BASIC	HUMIN	AMIDE	MONO-AMINO	BASIC
	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Leaves										
Mature										
C1 + D1 (Base) .....	0.0051	0.0169	0.0088	0.1010	0.4262	0.3684	0.1300	0.0689	0.2129	0.0777
C2 + C3 + D2 + D3 .....	0.0048	0.0060	0.0116	0.0896	0.3962	0.4235	0.1794	0.0561	0.3491	0.0648
C4 + D4 .....	0.0052	0.0068	0.0166	0.1666	0.6062	0.6930	0.2608	0.0861	0.7478	0.0610
C5 + D5 (Tip) .....	0.0079	0.0110	0.0195	0.2146	0.6382	0.7806	0.3850	0.1307	0.7880	0.1593
Active										
E1 + F1 (Base) .....	0.0073	0.0375	0.0124	0.1172	0.4139	0.4278	0.1663	0.0976	0.2713	0.0525
E2 + F2 .....	0.0024	0.0060	0.0070	0.0708	0.3364	0.2682	0.1400	0.0393	0.3080	0.0467
E3 + F3 .....	0.0075	0.0032	0.0144	0.1112	0.5806	0.6056	0.2275	0.0595	0.6510	0.1365
E4 + F4 .....	0.0080	0.0072	0.0191	0.2306	0.5567	0.6930	0.2975	0.0695	0.8170	0.1960
E5 + F5 (Tip) .....	0.0138	0.0078	0.0216	0.1000	0.8080	0.8860	0.3180	0.0870	0.8840	0.1818
Stem										
A + C (Lower) .....	0.0034	0.1977	0.0218	0.0700	0.6790	0.6965	0.1575	0.0341	0.3045	0.0840
D + E (Upper) .....	0.0132	0.1500	0.0271	0.1170	0.4850	0.5600	0.0980	0.0328	0.3010	0.0420
Pedicule .....	0.0025	0.3572	0.0067	0.0416	0.3082	0.3255	0.0665	0.0441	0.1085	0.0315
Fruit										
Shell .....	0.0172	0.0032	0.0170	0.1136	0.2302	0.3010	0.1365	0.0444	0.2065	0.0560
Flesh .....	0.0064	0.0050	0.0141	0.0390	0.1853	0.2128	0.0672	0.0261	0.0854	0.0182
Crown .....	0.0086	0.0603	0.0190	0.1616	0.6162	0.6458	0.1575	0.0641	0.4102	0.0798
Roots .....	0.0072	0.1043	0.0281	0.1072	0.4084	0.3570	0.2135	0.0556	0.3255	0.0770

TABLE III

DISTRIBUTION OF DIFFERENT FRACTIONS OF INORGANIC, SOLUBLE ORGANIC AND INSOLUBLE ORGANIC NITROGEN IN THE TISSUES OF THE LEAVES, STEM, AND FRUIT OF *Ananas comosus* (L.) MERR. GROWN IN NITRATE SOLUTION CULTURES AND IN DARKNESS FOR FOUR MONTHS

PLANT SECTIONS	MILLIGRAMS PER GRAM OF FRESH TISSUE									
	INORGANIC N			SOLUBLE ORGANIC NITROGEN			INSOLUBLE ORGANIC N			
	AMMO- NIUM	NITRATE	GLUTA- MINE	ASPARA- GINE	MONO- AMINO	BASIC	HUMIN	AMIDE	MONO- AMINO	BASIC
Leaves	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Mature										
C1 + D1 (Base) .....	0.0024	0.0313	0.0079	0.0800	0.3324	0.3920	0.0735	0.0257	0.1624	0.0616
C2 + D2 (Trans.) .....	0.0020	0.0438	0.0069	0.0876	0.2390	0.2744	0.1128	0.0387	0.1792	0.0560
Active										
E1 + F1 (Base) .....	0.0027	0.0438	0.0072	0.2184	0.4228	0.4172	0.0638	0.0202	0.1504	0.0560
E2 + F2 .....	0.0027	0.0141	0.0144	0.1664	0.5720	0.5796	0.0938	0.0209	0.1960	0.0648
E3 + F3 (Green) .....	0.0030	0.0047	0.0089	0.3074	0.6135	0.6468	0.1649	0.0371	0.2688	0.0924
Stem										
A + D (Lower) .....	0.0058	0.0727	0.0113	0.3160	0.6540	0.6468	0.1530	0.0429	0.2828	0.0896
E + F (Upper) .....	0.0172	0.0313	0.0621	0.9864	2.3572	1.8480	0.0666	0.0257	0.2184	0.0728
Peduncle .....	0.0086	0.1539	0.0419	0.6864	0.8468	0.6804	0.0534	0.0212	0.0840	0.0392
Fruit										
Shell .....	0.0057	0.0141	0.0225	0.2352	0.4928	0.4648	0.0802	0.0349	0.1792	0.0588
Flesh .....	0.0107	0.0078	0.0450	0.7492	1.0310	0.9184	0.0550	0.0259	0.1120	0.0420
Crown .....	0.0040	0.0063	0.0246	0.3336	0.7292	0.7280	0.0750	0.0327	0.1932	0.0616

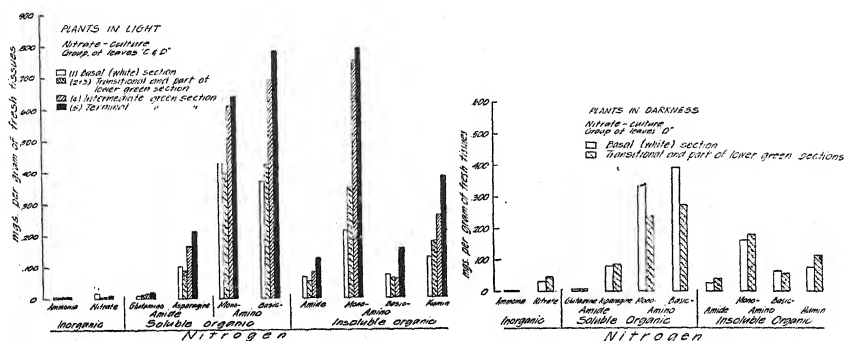


FIG. 8. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "C and D" groups of leaves of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "C and D" groups of leaves of pineapple sister shoots grown in solution cultures and kept in a darkened box for 4 months.

kept in light, however, were slightly greater than those in the plants kept in darkness.

4. The quantities of the various fractions of insoluble or protein nitrogen were generally greater in the plants kept in light than in those kept in darkness.

The data for the relatively young, but active, E and F leaf groups of the plants kept in light and in darkness, as presented in tables II and III and figures 9-A and 9-B, may be summarized as follows:

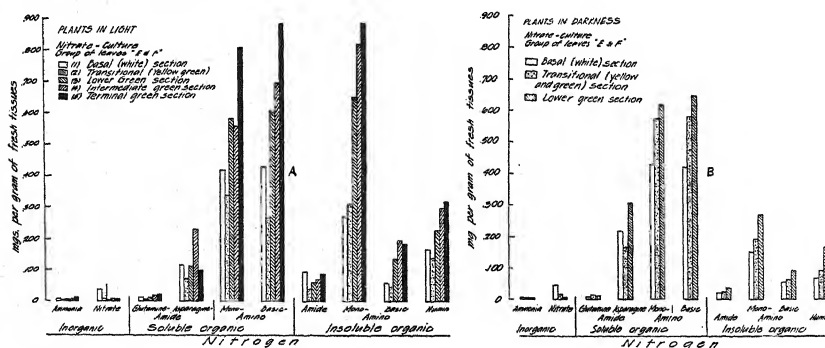


FIG. 9. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "E and F" groups of leaves of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in various sections of the "E and F" groups of leaves of pineapple sister shoots grown in solution cultures and kept in a darkened box for 4 months.

1. Ammonium occurred in traces. Nitrate was relatively abundant in the basal non-chlorophyllous and intermediate, or transitional, sections. In the chlorophyllous sections nitrate was present only in traces.

2. Glutamine was present in small amounts in the sections of the fruit from plants kept in light, but in considerably greater quantities in those of the fruit from the plants kept in darkness. Asparagine was present in appreciable quantities in the fruits from both the plants which had been kept in light and those kept in darkness. In the latter plants the quantities were greater than in the former.

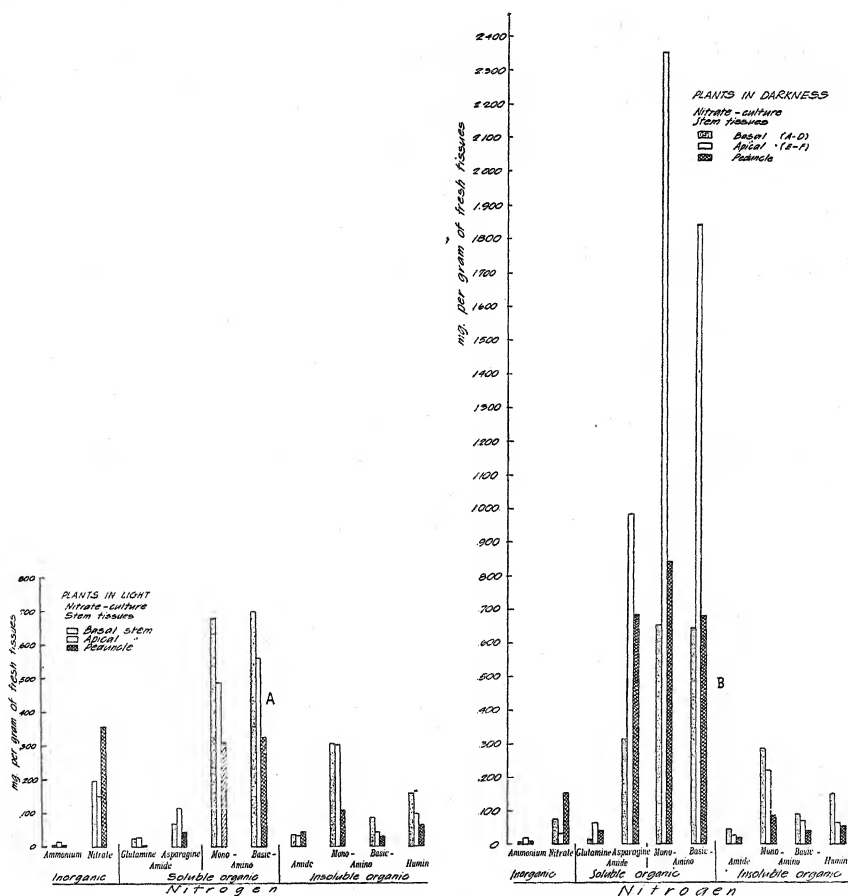


FIG. 10. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the lower and upper sections of the stem and peduncle of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the lower and upper sections of the stem and peduncle of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

3. The difference between the quantities of soluble mono-amino and basic nitrogen found in the plants kept in light and those kept in darkness was very small and possibly of no significance.

4. All fractions of insoluble organic or protein nitrogen were generally in greater quantities in the plants kept in light than in those kept in darkness.

The data for the fully mature basal section and for the young and immature apical section of the stem and peduncle of both the plants kept in light and in darkness, presented in tables II and III and figures 10-A and 10-B, show the following:

1. Ammonium was present in mere traces. Nitrate was relatively abundant in all three sections of the stem of both the plants kept in light and in darkness, its quantities being considerably greater in the sections of the former than in the latter plants.

2. Glutamine was present in greater quantities in the mature basal stem section and in considerably less quantities in the apical and peduncle sections of the stem of plants kept in light than those of plants kept in darkness. Asparagine was present in very small quantities in the plants kept in light when compared with the plants kept in darkness in which the quantities were exceedingly great.

3. The quantities of soluble mono-amino and basic nitrogen fractions were exceedingly great in the apical stem section and peduncle of the plants kept in darkness. In the basal section of the stem, the difference in the quantities of soluble mono-amino and basic nitrogen in the plants kept in light and those kept in darkness was comparatively small.

4. The mono-amino insoluble organic nitrogen fraction was present in appreciably greater quantities in the apical section of the stem of the plants kept in darkness.

The data for the distribution of nitrogen in the flesh, shell and crown sections of the fruits of plants kept in light and in darkness, presented in figures 11-A and 11-B, show the following:

1. Ammonium was present in small quantities in the fruit of both groups of plants. In the fruits of plants kept in light, the quantities were slightly greater than in those kept in darkness. The quantities of nitrate were small in the shell and flesh sections of fruits of both groups of plants and in the crown of the plants kept in darkness. In the crown of the plants kept in light nitrate was found in appreciable quantities.

2. The quantities of glutamine, except in the flesh section of fruits of the plants kept in darkness where it was found in appreciable amounts, was small in all other fruit sections of both groups of plants. Asparagine was present in very large amounts in all three sections of fruit from both groups of plants, although in greater quantities in the flesh of the fruit from the

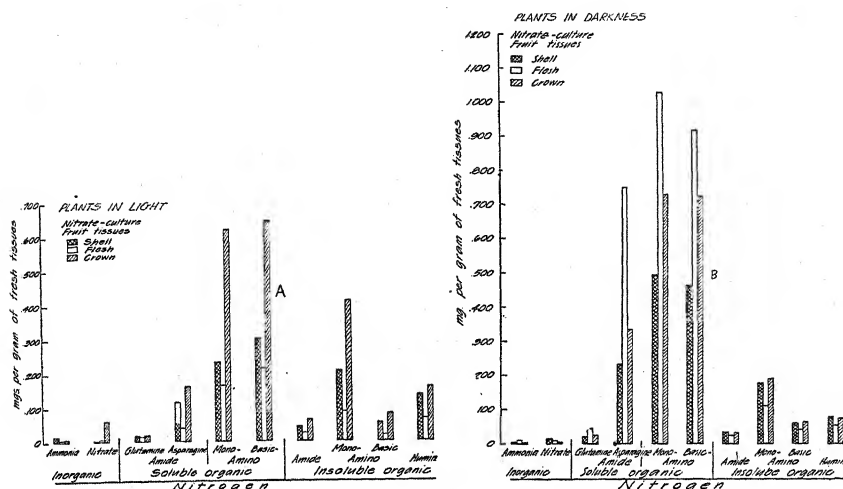


FIG. 11. A, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the flesh, shell and crown of the fruit of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of different fractions of inorganic, soluble organic, and insoluble organic nitrogen in the flesh, shell and crown of the fruit of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

plants kept in darkness. The quantities of asparagine were exceedingly small in the flesh section of fruits of the plants kept in light but in appreciably greater quantities in the shell and crown sections of the fruit from the same group of plants.

3. The quantities of soluble mono-amino and basic nitrogen were considerably greater in the sections of the fruit from plants kept in darkness than those kept in light.

4. Insoluble organic or protein nitrogen was, with a few exceptions, present in greater quantities in the fruits of plants kept in light than those kept in darkness.

The data for the distribution of nitrogen in the roots of the plants kept in light, as presented in table II, show the following:

1. Ammonium nitrogen was present only in traces while nitrate was in comparatively large amounts. The relatively high concentrations of nitrate and small amounts of ammonium in pineapple roots have been amply discussed in former studies (7).

2. With respect to the distribution of the various fractions of organic nitrogen, only the amounts of humin nitrogen were larger in the present than in our former studies, a condition which may be attributed either to a more advanced chronological age and physiological maturity of the tissues or to some unsuspected modification of our technique.



No comparable data were available for plants of the series kept in darkness because the roots were weak and by the time of harvest many had died.

#### DISTRIBUTION OF SUGARS

Table IV and figures 12-A and 12-B presenting the distribution of sucrose, reducing, and total sugars in the various sections of the leaves, stem, and fruit of the plants of both series show the following:

1. The amounts of reducing and of total sugars in the different leaf sections of both lots of plants showed no outstanding differences. In certain cases the leaf sections of the plants kept in darkness contained larger amounts of both reducing and total sugars than those of the plants grown in light. The cause for this is unknown.

TABLE IV

PERCENTAGE REDUCING, TOTAL SUGARS, AND SUCROSE, ON A FRESH WEIGHT BASIS, IN DIFFERENT SECTIONS OF PINEAPPLE LEAVES, STEM, AND FRUIT OF TWO SETS OF PINEAPPLE PLANTS, ONE EXPOSED TO LIGHT AND THE OTHER KEPT IN DARKNESS SINCE AN EARLY STAGE OF FRUIT DEVELOPMENT

PLANT AND FRUIT SECTIONS	IN LIGHT			IN DARKNESS		
	TOTAL	REDUCING	SUCROSE	TOTAL	REDUCING	SUCROSE
Leaves	%	%	%	%	%	%
Mature						
C1 + D1 (Base) .....	2.27	1.74	0.53	2.52	2.33	0.19
C2, 3 + D2, 3 .....	2.40	1.74	0.66	2.56	2.29	0.27
C4 + D4 .....	2.91	1.88	1.03	.....	.....	.....
C5 + D5 (Tip) .....	3.09	1.97	1.12	.....	.....	.....
Active						
E1 + F1 (Base) .....	2.33	1.82	0.51	1.93	1.72	0.21
E2 + F2 .....	2.36	1.95	0.41	2.58	2.38	0.20
E3 + F3 .....	1.77	1.01	0.76	2.58	2.35	0.23
E4 + F4 .....	2.07	1.03	1.04	.....	.....	.....
E5 + F5 (Tip) .....	2.59	1.28	1.31	.....	.....	.....
Stem						
A + C (Lower) .....	1.77	1.19	0.58	1.16	0.92	0.24
D + F (Upper) .....	2.19	1.54	0.65	0.75	0.45	0.30
Peduncle .....	3.30	1.46	1.84	1.24	1.12	0.12
Fruit						
Shell .....	6.08	2.25	3.83	1.34	1.14	0.20
Flesh .....	9.66	2.80	6.88	1.47	1.40	0.07
Crown .....	2.16	1.52	0.64	0.67	0.67	0.00
Roots .....	0.44	0.23	0.21	0.05	0.05	0.00

2. Sucrose, although usually found in relatively small amounts in the leaves, was present, comparatively, in considerably greater quantities in the leaves of the plants kept in light than in the corresponding sections of those of the plants kept in darkness.

3. The amounts of both reducing sugars and sucrose were consistently larger in the stem of the plants kept in light than in those kept in darkness.

The difference between the quantities of sugars in tissues of the two lots of plants was greater for the younger, upper, tissues of the stem (D-E section) and for the peduncle, than for the more aged tissues of the lower (A-C) stem section. Sucrose, being possibly a storage product (6), was practically absent in the peduncle and in the fruit tissues of the plants kept in darkness, but was very abundant in the same tissues of the plants kept in light.

The presence of relatively large amounts of reducing sugars in the leaves of the plants kept in darkness cannot be explained satisfactorily.

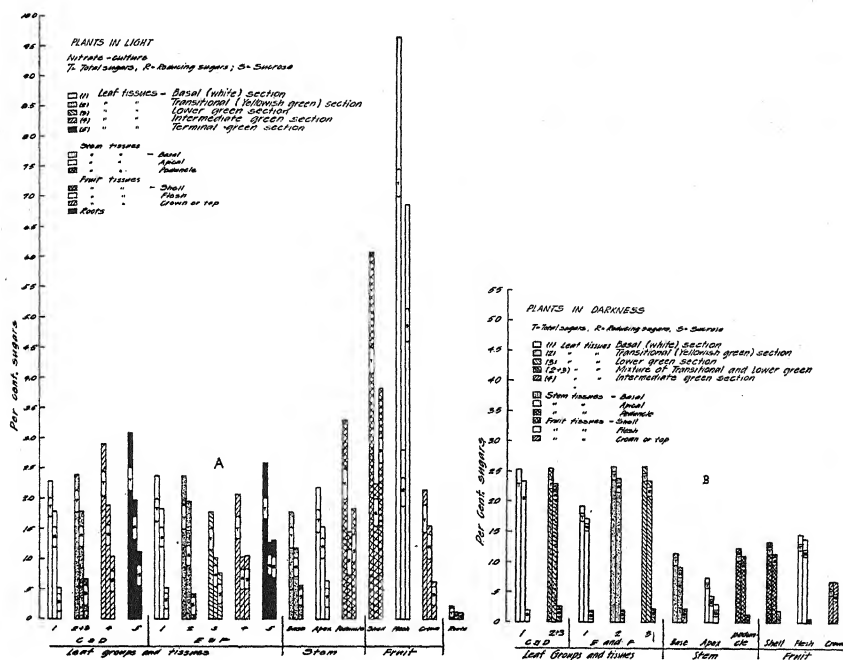


FIG. 12. A, distribution of reducing, total sugars and sucrose in different sections of leaf groups C, D, E, and F, and of the stem, fruit, and roots of pineapple sister shoots grown in solution cultures and exposed to greenhouse light for 5 months. B, distribution of reducing, total sugars, and sucrose in different sections of leaf groups C, D, E, and F, and of the stem, fruit, and roots of pineapple sister shoots grown in solution cultures and kept in darkness for 4 months.

### Discussion

The foregoing data show that the chemical composition of the tissues of pineapple plants and fruits kept in darkness differs considerably from that of plants kept in light.

Pineapple fruits grown in darkness contain very small amounts of sugars and organic acids when contrasted with similar fruits grown in light. The acidity of the flesh tissues of fruits grown either in light or in darkness

did not differ as much as that of the shell tissues. The greater acidity of the shell tissues of the fruits grown in light was probably due to the presence of greater amounts of carbohydrates, probably synthesized by the chlorophyllous tissues of the shell. It is also possible that the greater differences in acidity of the shell than of the flesh tissues in the two sets of fruits were due to the translocation of smaller amounts of sugars to the tissues of the shell than to those of the flesh of the fruits kept in darkness. Considering the fact that the chlorophyllous tissues of the shell of the fruits grown in darkness were unable to synthesize carbohydrates in darkness, and also that they are located at a greater distance from the core (the main artery for sugar supply) than the flesh, the greater difference in the acidity of shell than of flesh tissues as mentioned above, can be explained. Certain unpublished data by the authors have shown that extremely low carbohydrate supplies are often associated with the production of very small amounts of organic acids, in both the leaves and fruits of pineapple plants. In contrast with the above statement, it was observed in certain other unpublished experiments, that the amounts of organic acids in the fruit tissues of plants placed in darkness increased to very high levels if the fruits had approximately completed growth, but had not yet ripened at the time the plants were subjected to darkness. In this particular experiment the carbohydrate content of the fruits was relatively high.

With respect to assimilation and distribution of nitrogen, the data show that pineapple plants kept in darkness for a period of four months accumulated in the upper section of the stem, in the peduncle and in the flesh of the fruit, great quantities of soluble organic nitrogen consisting of asparagine and of various amino acids; the latter are reported in this paper collectively as mono-amino and basic nitrogen. Protein was present only in slightly greater amounts in the stem of the plants exposed to light than in those kept in darkness. Nearly all fractions of protein were from two to four times greater in the leaves of the plants exposed to light than in those kept in darkness.

The data show, in general, that there was a tendency for soluble organic nitrogen to accumulate in greater quantities in the non-chlorophyllous, and particularly in the meristematic regions, than in the highly differentiated chlorophyllous tissues of the plants kept in darkness. In the chlorophyllous sections of the leaves and in the lower section of the stem (composed mostly of thoroughly developed and mature tissues) the accumulation of soluble organic nitrogen was not as great as in the upper stem sections.

In the non-chlorophyllous sections of the leaves and fruit and in the upper section of the stem of the plants kept in darkness, the quantity of protein decreased either because its synthesis was inhibited through the lack of sufficient carbohydrates, or its disappearance was favored by enzymic hydrolysis.

In the chlorophyllous and in the more mature tissues of the same organs, protein was present in greater quantities either because its disappearance through enzymic hydrolysis was partially inhibited, or its synthesis was favored, to a certain extent, by the presence of carbohydrates.

Tables V and VI and figures 13 and 14, presenting the distribution of

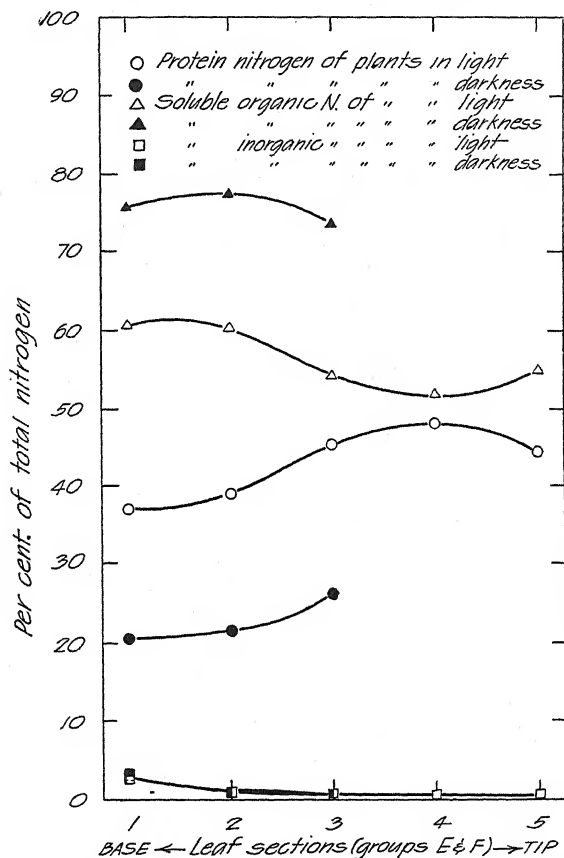


FIG. 13. Percentage distribution of total inorganic, soluble organic, and protein nitrogen in different sections of the E and F groups of leaves of pineapple sister shoots grown in nutrient solutions and exposed either to light or kept in darkness.

inorganic, soluble organic, and protein (insoluble organic nitrogen) on a percentage basis, show some very interesting relationships between the various nitrogen fractions in the two different lots of plants.

In sections 1 and 2, *i.e.*, the basal sections, of the mature C and D leaf groups, quantities of soluble organic nitrogen were 1.285, and protein 1.605 times, greater in the plants exposed to the light in the greenhouse than in those kept in the darkened box.

In sections 1, 2, and 3 of the younger and active E and F leaf groups, quantities of soluble organic nitrogen were 1.300, and protein 0.559 times, greater in the plants kept in darkness than in those exposed to light. If the same data are compared, however, on a percentage basis, as in figure 13, and not on a basis of actual amounts of nitrogen, slightly different results are

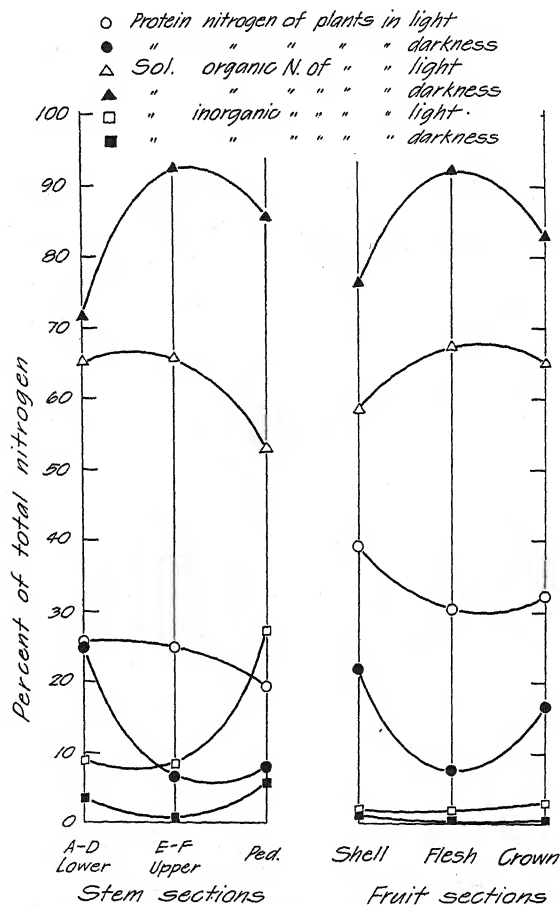


FIG. 14. Percentage distribution of total inorganic, soluble organic, and protein nitrogen in different sections of the stem and in the flesh, shell, and crown of the fruit of pineapple sister shoots grown in nutrient solutions and either exposed to light or kept in darkness.

obtained because of the difference in the amounts of nitrogen absorbed by the two lots of plants. The values for soluble organic, but not for protein nitrogen, were higher for the sections of the younger E and F leaf groups than for the more mature C and D groups of the plants kept in darkness, whereas the opposite condition prevailed in those exposed to light. The data,

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, INSOLUBLE ORGANIC, AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT TISSUES OF PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING NITRATE AND EXPOSED TO SEASONAL LIGHT UNDER GREENHOUSE CONDITIONS FOR FIVE MONTHS, TOGETHER WITH PERCENTAGE VALUES FOR THE SAME AND FOR THEIR FRACTIONS

PLANT SECTIONS	INORGANIC NITROGEN				SOLUBLE ORGANIC NITROGEN						INSOLUBLE ORGANIC NITROGEN						TOTAL N	
	AMMONIUM	Ni-TRACE	TOTAL	%	GLUTAMINE	ASPARAGINE	MONO-AMINO		BASIC	TOTAL	TOTAL	AMIDE	MONO-AMINO	BASIC	HUMIN	TOTAL		TOTAL
			TOTAL				%	%										
Leaves																		mg.
Mature																		%
C1 + D1 (Base) ...	23.0	77.0	0.002	1.8	0.9	11.2	47.2	40.7	0.904	63.6	14.1	43.5	15.9	26.5	0.490	34.6	1.416	
C2 + D2 .....	44.5	55.5	0.011	0.7	1.2	9.7	43.1	46.0	0.921	58.2	8.7	54.0	10.0	27.3	0.649	41.1	1.581	
C4 + D4 .....	43.5	56.5	0.012	0.4	1.1	11.2	40.9	46.8	1.482	53.9	6.9	59.6	12.8	20.7	1.256	45.7	2.750	
C5 + D5 (Tip) .....	7.5	92.5	0.019	0.2	1.0	12.9	38.6	47.5	1.653	52.8	8.9	54.0	10.9	26.4	1.463	47.0	3.135	
Active																		
E1 + F1 (Base) ...	16.4	83.6	0.045	2.7	1.1	12.1	42.6	44.2	0.971	60.6	16.6	46.3	9.0	28.1	0.588	36.7	1.604	
E2 + F2 .....	28.5	71.5	0.008	0.7	0.9	13.6	40.9	44.7	0.823	60.3	7.4	57.8	8.8	26.0	0.534	39.0	1.365	
E3 + F3 .....	70.1	29.9	0.011	0.7	0.8	5.6	45.8	47.8	1.268	53.8	5.5	60.5	12.7	21.3	1.075	45.5	2.357	
E4 + F4 .....	52.7	47.3	0.015	0.5	1.4	15.3	37.1	46.2	1.499	51.8	5.0	59.2	14.2	21.6	1.380	47.7	2.895	
E5 + F5 (Tip) .....	63.9	36.1	0.022	0.5	1.2	5.5	44.3	49.0	1.816	55.0	5.9	60.1	12.9	21.1	1.471	44.5	3.308	
Stem																		
A + D (Lower) ...	1.8	98.2	0.201	8.9	1.4	4.8	46.3	47.5	1.467	65.3	5.9	52.5	14.5	27.1	0.580	25.8	2.349	
E + F (Upper) ...	8.0	92.0	0.163	8.6	2.3	9.8	40.8	47.1	1.189	66.3	6.9	63.5	8.9	20.7	0.474	25.1	1.885	
Peduncle .....	0.8	99.2	0.360	27.8	0.9	6.1	45.3	47.8	0.682	52.8	17.6	43.5	12.6	26.3	0.251	19.4	1.292	
Fruit																		
Shell .....	84.3	15.7	0.020	1.8	2.5	17.2	34.8	45.5	0.662	58.8	10.0	46.5	12.7	30.8	0.443	39.4	1.126	
Flesh .....	56.2	43.8	0.011	1.8	3.1	9.1	38.4	49.4	0.431	67.4	13.9	43.3	9.1	33.7	0.197	30.8	0.640	
Crown .....	12.5	87.5	0.069	3.0	1.3	11.2	42.7	44.8	1.443	65.0	9.0	57.8	11.2	22.0	0.712	32.0	2.223	
Roots .....	6.7	93.3	0.112	6.7	3.1	11.9	45.3	39.7	0.901	53.4	8.3	48.6	11.5	31.6	0.672	39.9	1.684	

MILLIGRAMS OF INORGANIC, SOLUBLE ORGANIC, AND TOTAL NITROGEN PER GRAM OF FRESH WEIGHT IN THE DIFFERENT TISSUES OF PINEAPPLE PLANTS GROWN IN SOLUTION CULTURES CONTAINING NITRATE AND KEPT IN DARKNESS FOR FOUR MONTHS, TOGETHER WITH PERCENTAGE VALUES FOR THE SAME AND FOR THEIR FRACTIONS

PLANT SECTIONS	INORGANIC NITROGEN				SOLUBLE ORGANIC NITROGEN						INSOLUBLE ORGANIC NITROGEN					TOTAL N
	AMMONIUM	NITRATE	TOTAL	TOTAL	GLUTAMINE	ASPARAGINE	MONO-AMINO	BASIC	TOTAL	TOTAL	AMIDE	MONO-AMINO	BASIC	HUMIN	TOTAL	
	%	%	mg.	%	%	%	%	%	mg.	%	%	%	%	%	mg.	%
Leaves																mg.
Mature																
C1 + D1 (Base) ...	5.0	95.0	0.034	2.9	0.9	9.9	40.9	48.3	0.812	69.5	8.0	50.15	19.15	22.7	0.323	27.6
C2 + D2 .....	4.2	95.8	0.046	4.3	1.1	14.9	39.3	45.2	0.608	58.5	10.0	46.3	14.5	29.2	0.387	37.2
Active																
E1 + F1 (Base) ...	6.4	93.6	0.047	3.3	1.0	20.0	39.7	39.3	1.066	76.0	7.0	51.8	19.3	22.0	0.290	20.7
E2 + F1 .....	16.0	84.0	0.017	0.9	1.1	12.5	42.9	43.5	1.332	77.3	5.6	51.1	17.3	25.0	0.376	21.8
E3 + F3 (Green) ...	39.0	61.0	0.008	0.2	0.5	19.5	38.9	41.1	1.577	73.3	6.6	47.8	16.4	29.2	0.563	26.3
Stem																
A + D (Lower) ...	7.2	92.8	0.079	3.4	0.8	19.4	40.2	39.6	1.628	71.6	7.6	49.8	15.7	26.9	0.508	25.0
E + F (Upper) ...	35.5	64.5	0.049	0.8	1.0	18.8	45.0	35.2	5.254	92.4	6.7	57.0	19.0	17.3	0.384	6.8
Peduncle .....	5.5	94.5	0.163	6.1	1.7	30.5	37.6	30.2	2.256	86.2	10.7	42.5	19.8	27.0	0.198	7.7
Fruit																
Shell .....	29.0	71.0	0.020	1.3	1.8	19.3	40.6	38.3	1.215	76.5	9.9	50.8	16.6	22.7	0.353	22.2
Flesh .....	58.0	42.0	0.019	0.3	1.7	27.2	37.6	33.5	2.744	91.8	11.1	47.7	17.8	23.4	0.235	7.9
Crown .....	38.8	61.2	0.010	0.4	1.4	18.4	40.2	40.0	1.815	83.0	9.0	53.3	17.0	20.7	0.363	16.6

if our interpretation is correct, indicate that, in the absence of light, protein synthesis and accumulation were interrupted to a greater degree in the sections of the younger E and F leaf groups than in those of the older C and D leaf groups.

The percentage distribution of soluble organic and protein nitrogen in the stem sections of the plants of the two different treatments presents very sharp contrasts. The amounts of soluble organic nitrogen in the mature lower A-D sections of the stem of the plants kept in darkness were 1.110 times greater than those in light. There was hardly any difference between the percentage amounts of protein nitrogen in the same stem sections of the two lots of plants.

In the younger tissues of the upper D-E section of the stem the percentage of soluble organic nitrogen was 66.3 for the plants exposed to light and 92.4 for those kept in darkness. The percentage value for protein nitrogen for the former plants was 25.1 and for the latter group 6.8. If we consider, however, the actual amounts of soluble organic nitrogen in the upper D-E stem section, we find 1.189 mg. per gram of fresh weight for the plants exposed to light and 5.254 mg. per gram of fresh weight for those kept in darkness, or 4.42 times larger amounts for the latter lot. The actual amounts of protein nitrogen in the upper D-F stem section were 0.474 mg. per gram of fresh tissues for the plants exposed to light and 0.384 mg. per gram of fresh tissues in corresponding sections of plants kept in darkness, or only 1.235 times greater quantities in favor of the former lot. All such comparisons show that protein did not accumulate in the tissues in amounts equal to those of soluble organic nitrogen. The relatively small difference of 0.090 mg. of nitrogen between the amounts of protein nitrogen in the plants exposed to light and in those kept in darkness suggests that very small amounts of protein underwent enzymic hydrolysis and thereby increased the quantities of soluble organic nitrogen. Consequently, the accumulations of soluble organic nitrogen may have resulted from directly assimilated inorganic (nitrate) nitrogen without further conversion to protein, and not from hydrolyzed proteins. The data suggest that the processes for the conversion of soluble organic to protein nitrogen were retarded in the young, or upper D-F, stem section of the plants kept in darkness because these tissues lacked sufficient amounts of available carbohydrates and possibly other substances essential for protein synthesis. As is shown in table I and figure 2, the cessation of additional growth may have resulted in the accumulation of great quantities of soluble organic nitrogen. This was possibly caused by the inability of the tissues to synthesize protein in the absence of carbohydrates, etc., in the plants kept in darkness.

We do not wish, however, to give the impression in the above statement that protein hydrolysis by proteoclastic cellular enzymes is not a



regular feature of nitrogen catabolic processes. Our interpretation of the data is that, under the nutritional conditions of our experiments, where an ample external supply of inorganic nitrogen was available, the plants absorbed some of this supply and assimilated it only to the stage of soluble organic nitrogen and that, in the presence of great amounts of soluble organic nitrogen, protein hydrolysis was partly inhibited. This fact is well brought out by the examination of the values for the actual amounts and percentage values of protein nitrogen in the tissues of the mature lower (A-C) stem section. These values are approximately the same for the plants of both series, indicating that the effects of enzymic hydrolysis on protein catabolism were insignificant.

The amounts of soluble organic and protein nitrogen in the chlorophyllous sections of the leaves of groups C-D, which were dead after the plants had been kept for four months in continuous darkness, are presented in table VII. The values presented in this table, when contrasted with those for total soluble and insoluble organic nitrogen of comparable sections, as presented in table V, show that approximately two-thirds

TABLE VII

MILLIGRAMS OF SOLUBLE ORGANIC AND PROTEIN NITROGEN PER GRAM OF FRESH TISSUE  
IN THE DEAD CHLOROPHYLLOUS SECTIONS 4 AND 5, OF LEAF GROUPS C-D  
AND E-F OF PLANTS KEPT IN DARKNESS FOR FOUR MONTHS

PLANT SECTIONS	NITROGEN			
	SOLUBLE ORGANIC	PROTEIN	TOTAL	RATIO OF PROTEIN TO SOLUBLE ORGANIC
	mg.	mg.	mg.	
Leaves				
C-D <sub>4+5</sub> .....	0.480	0.785	1.285	1.64
E-F <sub>4+5</sub> .....	0.430	0.745	1.175	1.73

of the total soluble organic and one-half of the protein nitrogen migrated from the dead sections of the leaves to the stem. This assumption is made on the supposition that large quantities of soluble organic and protein nitrogen existed in the chlorophyllous sections of the leaves of the plants kept in the dark. Theoretically, the formation of great amounts of protein in the leaves of the plants kept in darkness is incompatible with the facts. We may assume, however, that organic nitrogen was present in both the plants kept in light and in darkness in approximately equal quantities and that the amounts of nitrogen represented by the difference between soluble organic and protein nitrogen in sections 4 and 5 of leaf groups C-D and E-F of the plants kept in light, as shown in table V, and that of the plants kept in darkness, as shown in table VII, all migrated into the tissues of the stem

of the plants kept in darkness. The amounts of migrated nitrogen represented by this difference are as follow: soluble organic: 1.155 (1.610 - 0.455); protein: 0.625 (1.390 - 0.765); total: 1.780. Thus the value for the amount of soluble organic nitrogen which migrated from the dead chlorophyllous sections of the leaves to the stem has been set at 1.155 mg. and for protein at 0.625 mg. of nitrogen per gram of fresh tissues. The total fresh weight of the dead sections was approximately 100 gm. and that of the stem, 120 gm. The influx of nitrogen from the leaves to the stem would have increased the original concentration, on the basis of these calculations, by 1.48 mg. per gram of fresh tissue. The average total soluble nitrogen concentration per gram of fresh tissue in the combined two sections of the stem and peduncle was 1.10 mg. for the plants growing in light and 3.04 mg. for the plants growing in the dark. If we subtract 1.48 mg. from 3.04 mg. we obtain 1.56 mg. which represents the concentration of soluble organic nitrogen per gram of fresh tissue in the combined sections of the stem and peduncle of the plants kept in darkness, after subtracting the amounts of nitrogen which moved to the stem and peduncle from the dead sections of the leaves. If all these assumptions and calculations are correct it is then safe to state that a great portion of the soluble organic nitrogen of the stem and peduncle of the plants kept in darkness was derived from the nitrogen reserves in the dead sections of the leaves.

The data for the chlorophyllous sections of the leaves and that for the upper stem indicate, to some extent, that protein hydrolysis had operated under the experimental conditions in the darkened box, liberating both carbohydrates and soluble nitrogen. It seems possible that the liberated carbohydrates were used to supply the energy requirements of the protoplasm and that soluble organic nitrogen accumulated. This view is in harmony with that of NIGHTINGALE (5) who suggests that the proteins of plants subjected to prolonged darkness are respired as a source of energy.

The percentage distribution of soluble organic and protein nitrogen in the peduncle of the plants of the two series followed, in spite of differences in the actual amounts, approximately the same trend as in the upper D-E stem sections.

The percentage distribution of different nitrogenous fractions in the tissues of the flesh, shell, and crown of the fruit varied considerably with the great diversity in function of these organs. The flesh, consisting entirely of non-chlorophyllous tissues, serves as a storage organ. The sepals and bracts of the shell, containing fair quantities of chlorophyll, act as photosynthetically assimilating organs until the time of the ripening of the fruit; while the petals, stamens, and style undergo all the morphological and metabolic processes which accompany flowering, growth, and ripening of the fruit. The crown may be compared morphologically and physiologically

to a small pineapple plant attached to a living substratum, but with its leaves and stem functioning autotrophically as plants grown in organic substrata.

The flesh of the fruit, being a storage organ, compares, in the order of the distribution of the fractions of soluble organic and protein nitrogen, more nearly with the stem and peduncle than with the leaves. Likewise, the shell compares, in this respect, more nearly with the leaves than with the stem or peduncle.

An examination of the actual amounts of insoluble nitrogen in the flesh of the fruit from the plants exposed to light shows that there were 0.197 mg. per gram of fresh tissues, while in the flesh of the fruits from plants kept in darkness the amount was 0.235 mg. per gram of fresh tissues or a difference of 0.038 mg. in favor of the latter series. This difference in favor of the plants in darkness is contrary to all expectations. It may be explained, however, by the fact that the weights of the fruits from the latter plants, as well as the sugars and organic acids content of their tissues, were considerably less than in the fruits of the plants grown in light. Consequently, the concentrations of nitrogen per volume of tissues were not distributed through as great a volume of tissues in the fruits from the plants in darkness as they were in those from the plants in light.

Table VIII, reporting the ratio values of soluble organic to protein

TABLE VIII

RATIO OF SOLUBLE ORGANIC TO INSOLUBLE ORGANIC NITROGEN IN DIFFERENT LEAF, STEM, AND FRUIT SECTIONS OF PINEAPPLE PLANTS GROWN EITHER IN THE SEASONAL LIGHT OF THE GREENHOUSE FOR FIVE MONTHS OR KEPT IN A DARK BOX FOR FOUR MONTHS

PLANT SECTIONS	IN DARKNESS	IN LIGHT
	<u>SOLUBLE ORGANIC-N</u> PROTEIN-N	<u>SOLUBLE ORGANIC-N</u> PROTEIN-N
Leaves		
Mature		
C1 + D1 (Base) .....	2.515	1.845
C2 + D2 .....	1.572	1.418
Active		
E1 + F1 (Base) .....	3.680	1.652
E2 + F2 .....	3.540	1.540
E3 + F3 (Green) .....	2.800	1.180
Stem		
A + D (Lower) .....	2.868	2.530
E + F (Upper) .....	13.670	2.510
Peduncle .....	11.400	2.320
Fruit		
Shell .....	3.400	1.495
Flesh .....	11.680	2.190
Crown .....	5.000	2.025

nitrogen, shows that, in the various sections of the plants exposed to light, the values for soluble organic nitrogen were from 1.18 to 2.53 times in excess of those for protein; whereas in plants kept in darkness, the former were from 1.572 to 13.670 times in excess of the latter. The ratio values of soluble organic to protein nitrogen were generally higher for the non-chlorophyllous and lower for the chlorophyllous sections of the plant and fruits.

In general, the amounts of soluble mono-amino and basic nitrogen fractions of the plants exposed to light were especially large when compared with those which were obtained in our former studies (7) with nitrate nutrition. On the basis of a similar comparison the mono-amino and basic fractions of insoluble organic or protein nitrogen were low. Total organic nitrogen values, however, were lower in the present than in our former studies (7). The low values obtained were possibly a result of the inhibiting effects of darkness on the synthesis of carbohydrates and other substances essential for protein production. It is also possible that a decreased rate of nitrogen absorption from the nutrient solution, as the result of the weakened condition of root systems of the plants of this series, may have been the cause for the small amounts of protein nitrogen found in the tissues. The greater quantities of soluble, and less of insoluble, nitrogen fractions in the plants of the present study in comparison to those of our former studies are probably attributable to differences in the chronological and physiological age of the two groups of plants. Amounts of humin nitrogen were comparatively large, being approximately twice as large as they were in the former studies (7). Although it is possible that the relatively large amounts of humin nitrogen are a result of the hydrolysis in the nitrogen fraction determinations, it is improbable that such a condition could have developed, because the same technique was employed in all cases. Humin nitrogen was found to increase with tissue age and maturity. It is, therefore, plausible to assume that the production of relatively great quantities of humin nitrogen was a result of the comparatively high rate of tissue maturation of leaf groups E and F during fruit development.

There was no outstanding difference in the distribution of either reducing or total sugars in the various sections of the plants of both series. Sucrose, however, although present in small quantities, was found in consistently greater quantities in the plants exposed to light than in those kept in darkness. This fact is brought out very well in table IX where the sucrose contents of the plants of the two series are contrasted on a ratio basis. The values of sucrose in the leaves were from 1.37 to 3.30 times greater for plants grown in light than for those grown in darkness. The peduncles of the plants grown in light contained 15.35 times more sucrose than the corresponding organs of the plants grown in darkness. The shell and flesh of the fruits of the plants kept in light contained 19.30 and 98.50 times, respectively, more sucrose than those kept in darkness.

TABLE IX

RATIO OF SUCROSE CONTENT OF DIFFERENT LEAF, STEM, AND FRUIT SECTIONS OF PLANTS KEPT IN THE SEASONAL LIGHT OF THE GREENHOUSE FOR FIVE MONTHS TO THAT OF THE SAME SECTIONS OF PLANTS KEPT IN DARKNESS FOR FOUR MONTHS

PLANT SECTIONS	S.P.L.*
	S.P.D.
Leaves	
Mature	
C1 + D1 (Base) .....	2.79
C2 + D2 .....	1.37
Young active	
E1 + F1 (Base) .....	2.43
E2 + F2 .....	2.05
E3 + F3 .....	3.30
Stem	
A + C (Lower) .....	2.42
D + E (Upper) .....	2.08
Peduncle .....	15.35
Fruit	
Shell .....	19.30
Flesh .....	98.50
Crown .....	.....

\* S.P.L. = Sucrose in plants kept in light.

S.P.D. = Sucrose in plants kept in darkness.

The data as presented above suggest that the relative quantities of sucrose, more so than those of reducing sugars, in the tissues of the stem and fruit, or of any other such storage organs, may serve as indices for the relative activity of the photosynthetic mechanism of plants under different light conditions. It is unfortunate that analytical data for starches, hemicellulose, and other carbohydrates are lacking. The data, however, regarding the distribution of sucrose in fruit and stem under the conditions of this experiment support the often made allegation that sucrose is a storage sugar. The small amounts of sucrose in the leaves of the plants kept in darkness may indicate either its utilization for synthetic or respiratory processes without subsequent replenishment of this substance, or hydrolysis and possible translocation to other parts of the plant.

The results support our view, in general, that light in relatively sufficient amounts favored protein and sucrose accumulation and darkness favored accumulations of soluble organic nitrogen.

A few comparisons between our data and those of VICKERY *et al* (9) show many similarities with respect to the distribution of organic solids, total organic acidity, etc., between pineapple and tobacco plants under similar light conditions.

### Summary

Two pairs of pineapple shoots obtained from two different mother plants were employed for these studies. The two members of each pair were of

approximately the same weight and degree of development. They were detached from their mother plants in February 1935 while at the red bud stage. After weighing they were placed in solution cultures in the greenhouse and allowed to produce roots. When all had developed vigorous and extensive root systems one of the two members from each pair of shoots was transferred to a dark box where it remained for the subsequent four months, while the other one was left in the greenhouse for five months. The fruits of the shoots kept in the darkened box ripened one month earlier than those in the greenhouse. At harvest, the leaves, stem and fruit were sectioned in order to separate senile from mature and the latter from young tissues and also chlorophyllous from non-chlorophyllous tissues. The results obtained were as follows:

1. The total weight of the plants, namely, the plant plus the fruit, growing in light increased 89 per cent. during the period of five months, while that of plants growing in darkness decreased 12.8 per cent. The increase in the former case was entirely due to the growth of the fruit, and the loss in the latter to the drying of the terminal section of the leaves. The weight of the fruit plus that of the crown was 3.28 times greater for the plants growing in light than for those growing in darkness.

2. The chemical properties of the fruits of the two series differed. The values for titratable acidity, reported as citric acid, of the shell was 3.27 times greater for the fruits from plants in light than for those in darkness.

3. The amounts of total soluble solids in the shell, determined refractometrically, were 3.15 times larger for the fruits from the plants in light than for the fruit from those in darkness. In the flesh they were 2.98 times larger for the former than for the latter series.

4. The distribution of different fractions of inorganic, soluble organic, and protein nitrogen differed considerably in different sections of the leaves, in the stem, and in the fruits of the plants exposed to light and those kept in darkness. Ammonium was present only in traces in nearly all plant sections. Nitrate was found in relatively large amounts in the peduncle, stem, and in the non-chlorophyllous basal sections of the leaves, in all other sections it was found in small amounts. Soluble organic nitrogen (consisting mostly of amino-, basic- and asparagine-nitrogen) was abundant in the meristematic and in the non-chlorophyllous sections of the leaves, while protein was most abundant in the chlorophyllous and highly differentiated tissues of the various organs. The ratio values of soluble organic to protein nitrogen were considerably greater for the leaf and stem tissues and fruits of the plants grown in darkness than for those of the plants grown in light, indicating that in the former series the assimilation of inorganic nitrogen had proceeded only to the stage of soluble organic but not to that of protein nitrogen.

5. No appreciable differences could be observed in the amounts of reduc-

ing sugars in the tissues of the two lots of plants. Sucrose, however, although present in small quantities in all vegetative organs, was consistently greater in the plants kept in light than in those kept in darkness. The leaves and stem of the plants exposed to light had from 1.4 to 3.3 times more sucrose than the same organs of the plants grown in darkness. The peduncle, shell, and flesh of the fruits of the plants in light contained 15, 19 and 99 times, respectively more sucrose than the corresponding sections of the fruits of the plants in darkness. The results have indicated rather conclusively that the accumulation of sucrose in storage organs under the minus-light conditions of these experiments was practically nil.

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# CHANGES IN THE COMPOSITION AND RATE OF GROWTH ALONG THE DEVELOPING STEM OF ASPARAGUS

C. W. CULPEPPER AND H. H. MOON

(WITH SIX FIGURES)

## Introduction

In view of the wide use of the tender immature stem of asparagus (*Asparagus officinalis* L.) as a food product and the rapid changes that occur in its composition at the stages of development desirable for table use, it appears advantageous to have a rather detailed history of the physical and chemical changes that take place during the entire period of its growth. A considerable amount of information concerning its growth and composition has been obtained but the story is complicated by many factors. The growth of the plant has been studied by BÜCHNER (3), WICHERS and TOLLENS (13), and WORKING (14). The composition of the stalk has been studied by BISSON, JONES, and ROBBINS (2), CULPEPPER and MOON (5), NIGHTINGALE and SCHERMERHORN (10), and MORSE (8, 9). The purposes of these several investigations differed widely.

No very thorough study of the composition of the developing stem in different zones as related to the rate of growth in these zones has been made. The purpose of this report is to present the results of some investigations bearing on this relationship.

## Materials and methods

The material employed in these studies was grown in the field at the Arlington Experiment Farm, Rosslyn, Va. Details of its culture have been given in an earlier report (6) upon the effect of temperature upon the elongation of the growing stem. Material for both studies was grown in the same plot with identical cultural treatment.

The rate of growth was determined by direct measurement of the increase in the length of short segments, marked out by suitable methods previously described (6). The measurements thus made have been recorded as the increase in length in centimeters of segments one cm. long during a time interval of 24 hours. The values are therefore read in cm. per cm. per 24 hours and are averages of 10 to 64 readings obtained as described in the earlier report (6).

The readings used in these studies were limited to those taken between 65° and 70° F., the average being close to 67.5° F. This, however, included readings made for many different zones along stalks of 14 different heights. The method of obtaining and recording the data will be clear from table I.

Each series of samples for chemical analysis was taken from 20 to 30



**TABLE I**  
 THE RATE OF GROWTH OF STALKS OF ASPARAGUS OF DIFFERENT HEIGHTS IN VARIOUS REGIONS IN THE GROWING ZONE.  
 EXPRESSED IN CM. OF GROWTH PER CM. PER 24 HOURS

HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH	HEIGHT OF GROW- ING ZONE	RATE OF GROWTH
TOTAL HEIGHT 15 CM.		TOTAL HEIGHT 45 CM.		TOTAL HEIGHT 75 CM.		TOTAL HEIGHT 105 CM.		TOTAL HEIGHT 135 CM.		TOTAL HEIGHT 165 CM.		TOTAL HEIGHT 175 CM.		TOTAL HEIGHT 175 CM.	
cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.	cm.
3.2	0.000	11.7	0.000	33.4	0.000	68.0	0.000	103.0	0.000	140.6	0.000	153.1	0.000	153.1	0.000
5.0	0.170	16.0	0.100	40.0	0.117	75.0	0.100	108.0	0.056	144.0	0.060	157.0	0.080	157.0	0.080
7.0	0.465	20.0	0.222	45.0	0.206	80.0	0.207	112.0	0.137	148.0	0.165	160.0	0.180	160.0	0.180
8.0	0.575	24.0	0.370	50.0	0.309	85.0	0.365	116.0	0.235	152.0	0.330	163.0	0.315	163.0	0.315
9.0	0.658	28.0	0.536	55.0	0.477	88.0	0.477	120.0	0.406	154.0	0.438	166.0	0.460	166.0	0.460
.....	.....	32.0	0.650	60.0	0.624	92.0	0.600	124.0	0.580	156.0	0.560	167.0	0.573	167.0	0.573
10.0	0.701	34.9	0.682	64.8	0.664	95.5	0.647	126.4	0.628	157.6	0.609	168.0	0.603	168.0	0.603
11.0	0.674	37.0	0.635	67.0	0.615	97.0	0.620	128.0	0.605	159.0	0.595	169.0	0.594	169.0	0.594
12.0	0.570	39.0	0.557	69.0	0.552	99.0	0.568	129.0	0.585	161.0	0.522	171.0	0.525	171.0	0.525
13.0	0.440	41.0	0.458	71.0	0.463	101.0	0.485	131.0	0.510	163.0	0.400	173.0	0.395	173.0	0.395
.....	.....	43.0	0.317	73.0	0.338	103.0	0.330	133.0	0.380	165.0	.....	.....	.....	.....	.....
15.0	0.000	45.0	0.000	75.0	0.000	105.0	0.000	135.0	0.000	.....	.....	.....	.....	.....	.....
TOTAL HEIGHT 25 CM.		TOTAL HEIGHT 55 CM.		TOTAL HEIGHT 85 CM.		TOTAL HEIGHT 115 CM.		TOTAL HEIGHT 145 CM.		TOTAL HEIGHT 175 CM.		TOTAL HEIGHT 175 CM.		TOTAL HEIGHT 175 CM.	
5.5	0.000	16.6	0.000	44.0	0.000	79.6	0.000	115.0	0.000	153.1	0.000	153.1	0.000	153.1	0.000
7.0	0.087	23.0	0.117	50.0	0.076	85.0	0.078	120.0	0.060	157.0	0.080	157.0	0.080	157.0	0.080
9.0	0.200	28.0	0.242	55.0	0.170	90.0	0.167	125.0	0.170	160.0	0.180	160.0	0.180	160.0	0.180
11.0	0.350	33.0	0.395	60.0	0.291	95.0	0.297	130.0	0.354	163.0	0.315	163.0	0.315	163.0	0.315
13.0	0.500	38.0	0.565	65.0	0.450	98.0	0.420	135.0	0.577	166.0	0.460	166.0	0.460	166.0	0.460
15.0	0.645	42.0	0.654	70.0	0.597	102.0	0.587	138.0	0.622	167.0	0.573	167.0	0.573	167.0	0.573
17.0	0.695	44.4	0.677	75.0	0.658	105.7	0.640	136.8	0.603	168.0	0.603	168.0	0.603	168.0	0.603
19.0	0.661	47.0	0.629	77.0	0.612	107.0	0.612	139.0	0.580	169.0	0.594	169.0	0.594	169.0	0.594
20.0	0.610	49.0	0.560	79.0	0.557	109.0	0.557	141.0	0.510	171.0	0.525	171.0	0.525	171.0	0.525
21.0	0.545	51.0	0.465	81.0	0.475	111.0	0.485	143.0	0.380	173.0	0.395	173.0	0.395	173.0	0.395
22.0	0.463	53.0	0.320	83.0	0.342	113.0	0.342	145.0	.....	.....	.....	.....	.....	.....	.....
25.0	0.000	55.0	0.000	85.0	0.000	115.0	0.000	145.0	0.000	175.0	0.000	175.0	0.000	175.0	0.000

TABLE I—(Continued)

[illegible]

stalks as closely comparable in size and behavior as it was possible to select from among those upon which measurements of growth rates were determined. These were collected between 9 and 10 A.M. and were cut about 2.5 cm. below the surface of the soil, taken to the laboratory, and cut transversely into segments of appropriate length. The length and position of the segments for stalks of different heights are given in table II. Branching usually began by the time stalks had reached a height of 100 cm. Where branches were present they were removed so that the samples consisted only of central axes.

In preparing the samples for analysis the segments were finely ground, duplicate 100-gm. samples weighed out, and 95 per cent. alcohol added in sufficient quantity to give a final concentration of 75 to 80 per cent. The material was then heated to boiling and the containers sealed and stored until the analyses were begun.

The nitrogen determinations were made upon separate samples which were dried rapidly in a current of warm air at 40° C. Total nitrogen was determined by the Gunning-Arnold method as modified to include nitrates.

The alcohol was decanted from the preserved material and passed through an extraction thimble. After adding alcohol twice and decanting each time, the residue was transferred to the extraction thimble which was then placed in a Soxhlet apparatus, and extracted about 8 hours with 95 per cent. alcohol to remove the last trace of alcohol-soluble materials. The extracts were combined, made up to volume, and aliquot portions taken for the determination of sugars, acids, and tannins. Sugars were determined by the volumetric permanganate modification of the Munson-Walker method (1).

The alcohol insoluble residue was dried, weighed, and duplicate portions taken for the estimation of the acid-hydrolyzable polysaccharides. These were determined by hydrolysis with hydrochloric acid as prescribed in the Official Methods (1).

Total astringency was determined by titration with N/20 potassium permanganate with indigocarmine as an indicator.

Total titratable acidity was determined by titration with N/10 sodium hydroxide with phenolphthalein as an indicator.

## Experimental results

### RATE OF ELONGATION ALONG THE STALK

Several observers have noted that the rate of growth along the stalk varies in different regions, and WORKING (14) has given a good account of this behavior. The data here presented are the averages of 116 stalks, and may be considered as representing the behavior of the field as a whole. Of course, the absolute rates will vary with the vigor of the plant, fertility of the soil,

the temperature and many other conditions. For a stalk of any given height the rate of growth at the apex is slow, but increases for a short distance downward from the apex to a maximum and then decreases again further toward the base to a point where it ceases. Table I gives the approximate height of the stalk at which it ceases to elongate, the height at which the rate is maximum, and the value of the maximum rate as well as a number of values at intermediate heights in the growing region, for plants 15 to 195 cm. in height. The progressive change in the rate of growth along the stalk is evident from the table but a clearer understanding of the manner of this change may be gained from figure 1. The values of the rates of growth

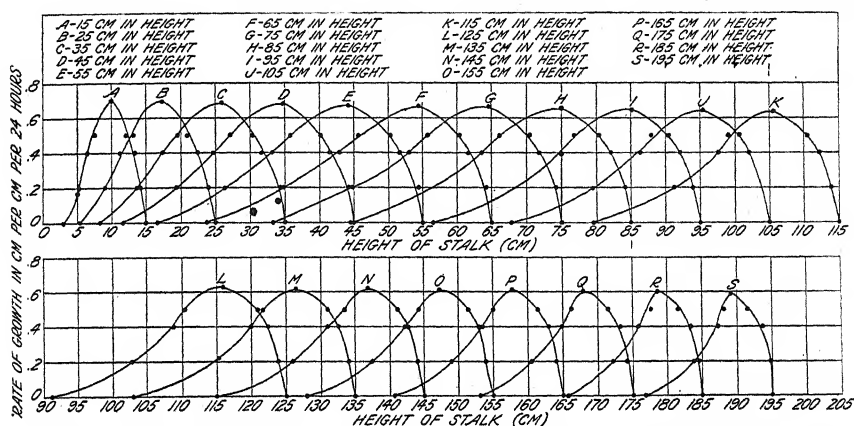


Fig. 1. The rate of growth of the stalks of asparagus at successive intervals from base to tip for stalks of various total heights.

represented by the ordinates are plotted against the corresponding heights of the growing segments of the stalk as abscissae for plants of many different total heights. A system of curves is thus formed, each curve of which represents the growth of a stalk of a particular height. Thus A represents the growth of a stalk 15 cm. high; B, one 25 cm. high; and so on at 10 cm. intervals until the stalk is 195 cm. in height. Each of the curves begins at a certain point on the abscissa at which the value of the ordinate is zero and ends at a point corresponding to the total height of the plant. Between the point on the abscissa at which the curve begins and the origin of the abscissa the rate of growth is obviously zero.

The curves show at once that the rate of growth in different zones along the stalk varies continuously from the lower point, where the growth ceases, to the tip. The form of the curve, however, is different for the plants of each particular height. For plants 15 cm. in height the curve rises rapidly to a maximum and then declines just as rapidly to the tip of the stalk. For those 65 to 75 cm. tall the curve rises gradually to a peak, then declines rapidly,

the decline being only a little less rapid than in the young stalks. For plants 195 cm. tall the curves rise gradually at first, then more sharply until a maximum is reached after which there is a rapid decline.

It is noted that the height of the point at which the stalk ceases to elongate is constantly being shifted upward as the plant becomes taller, but the rate does not parallel the rate of growth of the plant in height, being slower in the short, or young stalks, than in the high ones. The point of cessation of growth moves up the stalk slowly in the early stages of growth and then more rapidly as a height of 75 cm. or more is reached.

The point on the curve representing the zone of maximum rate of elongation shows that the rate of growth is maximum in a zone a short distance below the tip but this distance from the tip is not constant; it varies somewhat with the height of the stalk, being shortest in the youngest and the oldest stalks. The maximum rate of growth per unit length appears to be nearly constant for plants of all heights within the range studied. There is a tendency for the maximum rate to decline as the stalks become taller. The maximum is 0.7 cm. per cm. per 24 hours in stalks 15 cm. tall and 0.59 cm. per cm. for those 195 cm. tall.

It was pointed out in an earlier report (6) that the total amount of elongation occurring in 24 hours varies with the height of the stalk. Since it was found that the maximum rate of growth per unit length was nearly the same for stalks of all heights, it follows that differences in amount of elongation among stalks of different heights must be attributed for the most part to differences in the length of the portion that is elongating. The length of the elongating region varies with increasing height of the stalk, being about 11.7 cm. in stalks 15 cm. high; about 41.5 cm. in those 75 cm. high (in which it was at a maximum); and 18.3 cm. in those 195 cm. high. The height of the stalk in which the elongating region is of maximum length corresponds closely to that at which the total elongation is at a maximum, the most rapid rate of total elongation being in stalks 65 cm. high and the length of the growing region being maximum in those 75 cm. high.

#### COMPOSITION OF THE STALK AT DIFFERENT HEIGHTS

Variation in the growth rate along the stalks of asparagus raises the question as to what chemical changes are occurring in the different regions and how these are related to the growth rates. It was not feasible to make a sufficient number of replications of the analyses to procure enough data to permit curve smoothing as was done with the growth data, hence no attempt has been made to average the results.

The material used for analyses was necessarily taken from different stalks than those used in the growth measurements, but was as nearly identical as the writers could select. The possibility of a certain amount of sampling error, however, must be kept in mind.

Analyses were confined to the following constituents: total solids, sugars, total nitrogen, acids, tannins, and acid-hydrolyzable polysaccharides.

The results of the analyses are given in table II. The method of analyzing the growth data was not anticipated when the chemical samples were taken, and as a result the data for the composition and the data for the growth rates are not always recorded for stalks of identical heights or for identical positions on the stalk. Therefore, the relationship between the composition and the rate of growth is more easily comprehended if the curves obtained by plotting the data against the height of the segments are compared.

#### MOISTURE CONTENT AND GROWTH RATE

In figure 2 the percentage of total solids in successive segments along the

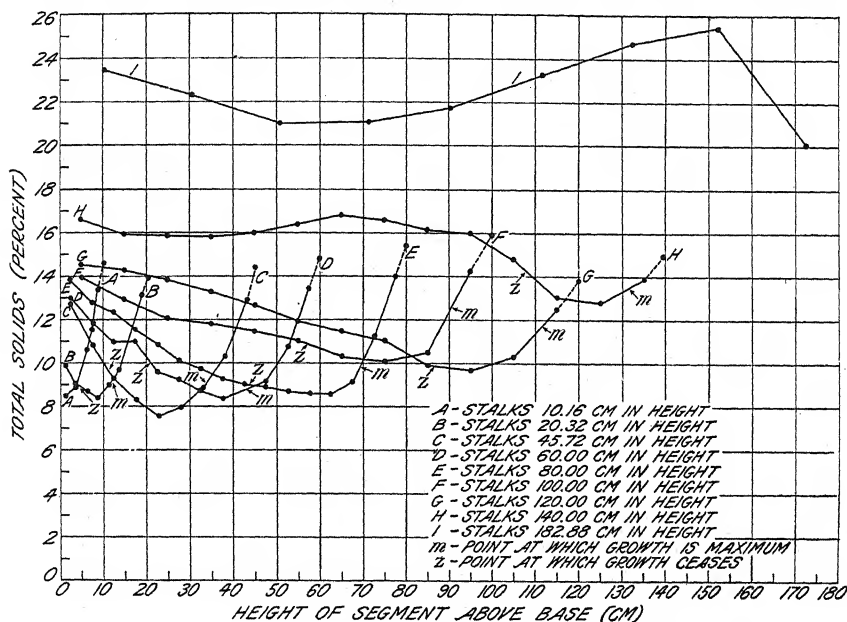


FIG. 2. The percentage of total solids in asparagus in successive segments along the stalk for stalks of various heights.

stalk is plotted against the height of the segment above the base for stalks of various heights. It is evident that there is a great variation in the solids content of stalks of different height as well as in different regions along the stalk. Also, increase in height of the stalk is accompanied by a progressive increase in total solids. In stalks 10.1 cm. high the solids are lowest at the base and increase rapidly toward the tip. In stalks 20.3 cm. high the solids in the basal segments are somewhat higher than in the base of the 10.1 cm. stalks but they decrease along the stalk to a point about 9 cm. above

TABLE II

COMPOSITIONS OF THE ASPARAGUS STALK AT DIFFERENT POINTS ALONG THE STALK FROM BASE TO TIP FOR STALKS OF VARIOUS HEIGHTS. EXPRESSED AS PERCENTAGE OF THE FRESH WEIGHT

DATE OF SAMPLING	TOTAL HEIGHT OF STALK	LENGTH OF SEGMENT ANALYZED	MEAN HEIGHT OF SEGMENT FROM BASE	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	TOTAL SOLIDS	REDUCING SUGARS	NON REDUCING SUGARS	TOTAL SUGARS	ACID HYDROLYZABLE POLYSACCHARIDES	TOTAL ASTRINGENCY	TITRATABLE ACIDITY CALCULATED AS CITRIC
	cm.	cm.	cm.	%	%	%	%	%	%	%	%	%
May 4	10.16	2.4	1.27	5.72	2.77	8.49	3.05	1.05	4.10	0.69	0.076	0.227
" "	"	"	3.81	5.46	3.38	8.84	2.81	1.17	3.98	0.76	0.072	0.243
" "	"	"	6.35	5.36	5.23	10.59	2.16	1.14	3.30	0.98	0.100	0.255
" "	"	"	8.89	5.06	8.15	13.21	1.07	1.67	1.74	1.23	0.161	0.290
May 4	20.32	2.5	1.27	5.74	4.15	9.89	3.08	1.08	4.16	1.25	0.070	0.163
" "	"	"	3.81	5.40	3.65	9.05	2.98	0.24	3.92	0.95	0.074	.....
" "	"	"	6.35	5.16	3.57	8.73	2.94	0.86	3.80	0.91	0.084	0.185
" "	"	"	8.89	4.92	3.45	8.38	2.60	0.92	3.52	0.80	0.084	0.185
" "	"	"	11.43	4.94	4.05	8.99	2.48	0.64	3.12	0.86	0.104	0.227
" "	"	"	13.97	4.94	4.69	9.63	2.10	0.80	2.90	0.93	0.127	0.234
" "	"	"	16.51	4.92	6.27	11.19	1.57	0.85	2.42	1.00	0.151	0.286
" "	"	"	19.05	4.58	8.54	13.12	0.05	0.24	0.29	0.97	0.239	0.306
May 12	45.72	5	2.54	6.56	6.20	12.76	3.63	1.31	4.94	1.75	0.081	0.202
" "	"	"	7.62	5.98	4.82	10.80	3.39	1.28	4.68	1.30	0.082	0.189
" "	"	"	12.70	5.40	3.84	9.24	3.06	1.04	4.10	1.03	0.085	0.189
" "	"	"	17.78	4.94	3.32	8.26	2.87	0.69	3.56	0.85	0.081	0.174
" "	"	"	22.86	4.62	2.94	7.56	2.68	0.66	3.34	0.66	0.098	0.189
" "	"	"	27.94	4.56	3.39	7.95	2.43	0.73	3.16	0.75	0.096	0.228
" "	"	"	33.02	4.54	4.30	8.84	2.11	0.75	2.86	0.82	0.100	0.270
" "	"	"	38.10	4.60	5.68	10.28	1.58	0.66	2.24	0.86	0.144	0.274
" "	"	"	43.18	4.48	8.42	12.90	0.10	0.20	0.30	0.80	0.217	0.300

TABLE II—(Continued)

DATE OF MPLING	TOTAL HEIGHT OF STALK	LENGTH OF SEG- MENT ANALYZED	MEAN HEIGHT OF SEG- MENT FROM BASE	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	TOTAL SOLIDS	REDUCING SUGARS	NON REDUCING SUGARS	TOTAL SUGARS	ACID HYDRO- LYZABLE POLYSAC- CHARIDES	TOTAL ASTRIN- GENCY	TITRATABLE ACIDITY CAL- CULATED AS CITRIC
	cm.	cm.	cm.	%	%	%	%	%	%	%	%	%
ay 16	60	5	2.5	6.05	6.96	13.01	2.31	0.87	3.18	2.00	0.050	0.282
"	"	"	7.5	5.43	6.38	11.81	2.46	0.63	3.09	1.81	0.046	0.282
"	"	"	12.5	5.13	5.85	10.98	2.50	0.43	2.93	1.49	0.056	0.229
"	"	"	17.5	5.11	5.88	10.99	2.16	0.73	2.89	1.35	0.064	0.261
"	"	"	22.5	5.08	4.55	9.63	1.88	0.82	2.70	1.08	0.066	0.249
"	"	"	27.5	5.28	3.96	9.24	2.21	0.41	2.62	0.98	0.076	0.260
"	"	"	32.5	5.20	3.56	8.76	2.30	0.33	2.63	0.94	0.073	0.315
"	"	"	37.5	4.93	3.39	8.32	2.11	0.33	2.44	0.87	0.080	0.315
"	"	"	42.5	4.80	3.82	8.62	2.04	0.19	2.23	0.95	0.097	0.327
"	"	"	47.5	4.80	4.37	9.17	1.73	0.44	2.17	0.84	0.104	0.352
"	"	"	52.5	4.88	5.86	10.74	1.30	0.43	1.73	0.78	0.132	0.302
"	"	"	57.5	4.82	8.61	13.43	0.20	0.29	0.49	0.74	0.184	0.324
ay 17	80	5	2.5	5.80	8.03	13.83	2.87	0.60	3.47	2.85	0.063	0.261
"	"	"	7.5	5.63	7.11	12.74	2.54	0.82	3.36	2.79	0.056	0.235
"	"	"	12.5	5.68	6.62	12.30	2.64	0.70	3.34	2.51	0.059	0.236
"	"	"	17.5	5.34	6.18	11.52	2.46	0.83	3.29	2.64	0.041	0.215
"	"	"	22.5	5.19	5.63	10.82	2.04	0.86	2.90	2.86	0.061	0.253
"	"	"	27.5	5.01	5.07	10.08	2.39	0.53	2.92	2.05	0.056	0.236
"	"	"	32.5	4.95	4.79	9.74	2.37	0.41	2.78	1.97	0.054	0.245
"	"	"	37.5	5.03	4.23	9.26	2.39	0.28	2.67	1.70	0.060	0.276
"	"	"	42.5	4.94	4.09	9.03	2.21	0.54	2.75	1.35	0.054	0.251
"	"	"	47.5	5.13	3.79	8.92	2.35	0.32	2.67	1.01	0.060	0.270
"	"	"	52.5	4.94	3.80	8.74	2.30	0.28	2.58	0.98	0.062	0.256
"	"	"	57.5	4.90	3.74	8.64	2.24	0.09	2.33	0.90	0.056	0.281
"	"	"	62.5	4.58	4.02	8.60	2.13	0.20	2.33	0.94	0.072	0.296
"	"	"	67.5	4.90	4.23	9.13	1.72	0.35	2.07	0.96	0.076	0.313
"	"	"	72.5	4.87	6.40	11.27	1.34	0.33	1.67	1.02	0.115	0.285
"	"	"	77.5	4.75	9.25	14.00	0.16	0.03	0.19	0.98	0.185	0.300



TABLE II—(Continued)

DATE OF SAMPLING	TOTAL HEIGHT OF STALK	LENGTH OF SEGMENT ANALYZED	MEAN HEIGHT OF SEGMENT FROM BASE	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	TOTAL SOLIDS	REDUCING SUGARS	NON REDUCING SUGARS	TOTAL SUGARS	ACID HYDROLYZABLE POLYSACCHARIDES	TOTAL ASTRINGENCY	TITRATABLE ACIDITY CALCULATED AS CITRIC
	cm.	cm.	cm.	%	%	%	%	%	%	%	%	%
May 18	100	10	5	6.04	7.84	13.88	3.16	0.72	3.88	2.68	0.046	0.201
" "	"	"	15	5.66	7.21	12.87	2.95	0.61	3.56	2.89	0.056	0.218
" "	"	"	25	5.45	6.57	12.01	2.70	0.70	3.40	2.90	0.060	0.215
" "	"	"	35	5.26	6.54	11.80	2.74	0.45	3.19	2.00	0.072	0.242
" "	"	"	45	5.42	6.14	11.56	2.44	0.60	3.04	2.31	0.080	0.261
" "	"	"	55	5.48	5.61	11.09	2.56	0.53	3.09	1.82	0.088	0.305
" "	"	"	65	5.12	5.17	10.29	2.72	0.28	3.00	2.00	0.076	0.236
" "	"	"	75	5.71	4.41	10.12	2.59	0.25	2.84	1.56	0.074	0.260
" "	"	"	85	5.38	4.62	10.50	2.33	0.19	2.52	0.95	0.100	0.327
" "	"	"	95	5.96	8.28	14.24	1.27	0.38	1.64	0.86	0.283	0.330
May 23	120	10	5	5.96	8.57	14.53	3.26	0.48	3.74	3.11	0.046	0.196
" "	"	"	15	5.84	8.45	14.29	3.16	0.44	3.60	3.00	0.052	0.242
" "	"	"	25	6.40	7.43	13.83	2.77	0.74	3.51	3.21	0.052	0.249
" "	"	"	35	6.32	7.03	13.35	2.83	0.44	3.27	2.96	0.056	0.201
" "	"	"	45	6.16	6.49	12.65	2.68	0.42	3.10	2.74	0.062	0.268
" "	"	"	55	5.09	6.90	11.99	2.60	0.28	2.88	2.27	0.058	0.222
" "	"	"	65	5.25	6.25	11.50	2.50	0.33	2.83	1.95	0.062	0.322
" "	"	"	75	5.09	5.98	11.07	2.42	0.38	2.70	1.92	0.064	0.235
" "	"	"	85	5.64	4.34	9.98	2.18	0.47	2.65	1.99	0.060	0.252
" "	"	"	95	4.70	5.00	9.70	2.23	0.37	2.60	0.94	0.088	0.402
" "	"	"	105	5.42	4.85	10.27	2.19	0.26	2.45	0.92	0.100	0.392
" "	"	"	115	5.53	6.93	12.46	1.44	0.11	1.55	0.96	0.220	0.320

TABLE II—(Concluded)

DATE OF AMPLING	TOTAL HEIGHT OF STALK	LENGTH OF SEG- MENT ANALYZED	MEAN HEIGHT OF SEG- MENT FROM BASE	SOLUBLE SOLIDS	INSOLUBLE SOLIDS	TOTAL SOLIDS	REDUCING SUGARS	NON REDUCING SUGARS	TOTAL SUGARS	ACID HYDRO- LYZABLE POLYSAC- CHARIDES	TOTAL ASTRIN- GENCY	TITRATABLE ACIDITY CAL- CULATED AS CITRIC
	cm.	cm.	cm.	%	%	%	%	%	%	%	%	%
une 8	140	10	5	5.50	11.10	16.60	3.07	0.75	3.82	2.31	0.040	0.252
" "	"	"	15	5.30	10.61	15.91	3.20	0.54	3.74	2.96	0.064	0.332
" "	"	"	25	5.76	10.06	15.82	2.18	0.52	3.70	3.00	0.076	0.356
" "	"	"	35	5.89	10.91	15.80	1.60	0.92	3.52	2.96	0.076	0.384
" "	"	"	45	5.60	10.41	16.01	1.78	0.72	3.50	2.85	0.091	0.376
" "	"	"	55	5.53	10.89	16.42	1.90	0.52	3.42	2.85	0.096	0.412
" "	"	"	65	5.51	10.30	16.81	1.57	0.73	3.30	2.92	0.104	0.404
" "	"	"	75	5.86	10.74	16.60	1.75	0.25	3.00	2.50	0.096	0.444
" "	"	"	85	5.68	10.50	16.18	1.56	0.26	2.82	2.71	0.120	0.450
" "	"	"	95	5.78	10.24	16.02	1.42	0.98	2.40	2.68	0.148	0.458
" "	"	"	105	5.72	10.10	14.82	1.36	0.65	2.01	2.47	0.144	0.444
" "	"	"	115	6.04	7.02	13.06	1.06	0.81	1.87	1.89	0.208	0.484
" "	"	"	125	6.44	6.36	12.80	1.00	0.48	1.48	1.78	0.280	0.430
" "	"	"	135	6.96	6.86	13.82	0.94	0.42	1.36	0.89	0.324	0.458
une 1	182.88	20.32	10.16	6.10	17.35	23.45	2.93	1.85	4.28	4.65	0.104	0.338
" "	"	"	30.48	5.80	16.54	22.34	2.68	1.20	3.88	4.17	0.141	0.342
" "	"	"	50.80	5.64	15.35	21.00	2.67	0.95	3.62	3.62	0.116	0.377
" "	"	"	71.12	5.64	15.44	21.08	2.34	1.02	3.36	3.54	0.128	0.393
" "	"	"	91.44	5.38	16.99	21.77	2.35	0.73	3.08	3.65	0.134	0.404
" "	"	"	111.76	5.12	18.12	23.24	2.34	0.72	3.06	3.99	0.128	0.443
" "	"	"	132.08	5.20	19.49	24.69	1.95	0.77	2.72	4.25	0.145	0.455
" "	"	"	152.40	5.04	20.39	25.43	1.64	0.80	2.44	4.61	0.172	0.500
" "	"	"	172.72	5.64	15.08	20.72	1.05	0.29	1.34	2.64	0.486	0.491

the base, then increase rapidly to the tip. In stalks 45.7 cm. tall the curve is distinctly U-shaped, the lowest solids content being about 23 cm. from the base, or about 20 cm. below the tip. In stalks of all heights up to 150 cm. the solids are lowest in some portion intermediate between the base and the tip. The zone of low solids is constantly being shifted upward as the stalks become taller. The solids content increases rapidly in the basal portion of the stalks as they increase in height; likewise segments immediately above the base continue to increase in solids content so that the length of the basal portion with a high solids content is constantly increasing. This results in a high solids content all along the stalks as they approach maturity. The curves are thus constantly changing form as the height of the stalk increases, being U-shaped in the young, then dipper-shaped, and finally more or less horizontal in the mature stalks.

It may be estimated from figure 2 that in stalks 60 cm. tall, the zone of elongation begins at about 20.5 cm. and reaches a maximum at about 49.5 cm. from the base, and as indicated in figure 3, curve d, the lowest solids content is found approximately 37 cm. from the base. Growth ceases at Z (fig. 2), and is at a maximum at M. The highest moisture content is found just above the point at which growth ceases and just below the point at which growth rate is at a maximum. In stalks 80 cm. tall the lower limit of growth is 38.5 cm. above the base and growth is maximum at about 69.5 cm. The highest moisture content is about 56 cm. above the base. The moisture content, then, is highest neither in the region in which growth is at its maximum nor at the lower limit of the growing region. If the two sets of stalks used for measurements of growth rate and chemical analysis are comparable, it must be concluded that the cell walls begin to thicken sufficiently to give a higher solids content in the stalk before the cells wholly cease to elongate.

It is evident that hydration is an important factor in the elongation of the stalk. At the tip of the stalk, cell division is very rapid but the cells enlarge slowly at first probably because the mechanism of water transport is as yet imperfectly formed. As soon as water is brought to the cells in quantity they enlarge very rapidly but soon after the transporting system is sufficiently formed to function adequately for the enlarging cells, its structural elements gradually become rigid and elongation ceases. These processes are constantly going on so that the region of activity is constantly being shifted upward.

#### SOLUBLE AND INSOLUBLE SOLIDS

The proportion of soluble to insoluble solids differs greatly in different zones along the stalk and varies somewhat in stalks of different heights. It may be noted from table II that the soluble solids are less variable than the insoluble. There is a tendency for the soluble solids to be highest at the base and lowest at the tip or in a zone somewhat below the tip. The difference in

the percentage of soluble solids between the old and young stalks is not very great and appears unimportant. The insoluble solids content changes very greatly from base to tip, being lowest in a zone between the region of maximum rate of growth and the lower limit at which growth occurs. At the base the insoluble solids are evidently composed very largely of structural materials and vary greatly in amount with the height of the stalk or the age of the tissues. The insoluble solids at the tip are obviously high in protein and their amount does not vary as widely as the insoluble content at the base.

#### TOTAL SUGARS AND GROWTH RATE

The results of the sugar analyses show (fig. 3) that the sugar content is, in general, fairly low, and varied considerably in the different series of

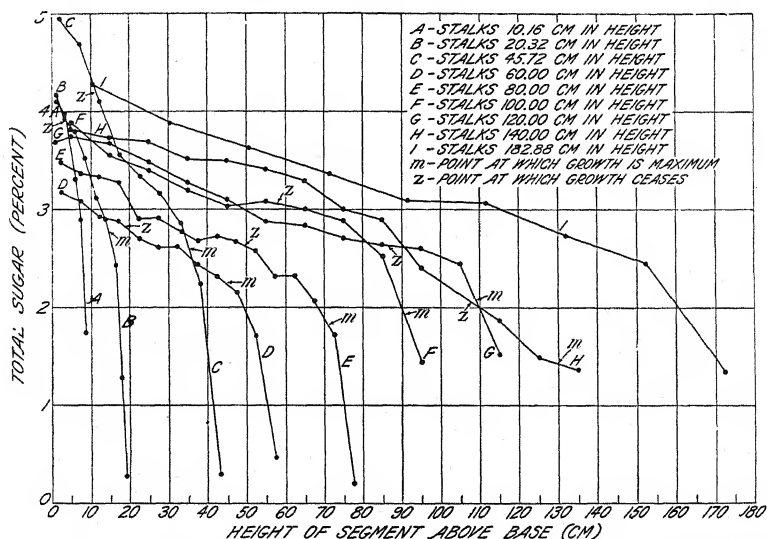


FIG. 3. Percentage of total sugar in asparagus in successive segments along the stalks of various heights.

stalks. This variation is probably due to sampling error, to differences in crown reserves, or to some variation in weather conditions at the time the samples were taken. The sugar content varied tremendously in different regions along the stalk, always being highest at the base and lowest at the tip. Sugar is apparently almost absent at the extreme tip of the growing stalk. The sugar content in the terminal segment of the stalks above 80 cm. in height is somewhat higher than in the terminal segments of the short stalks, probably because the segment analyzed was much longer and consequently included tissues far enough down the stalk to be somewhat higher in sugar than at the tip. The concentration gradient is that to be expected

where sugar is being transported from the root to the growing tip if diffusion plays a part in the process. As the stalks become taller the basal portion that is high in sugar increases in length so that the content at any specific height is constantly changing in an elongating stalk.

The letter M (fig. 3) indicates the region at which the growth is at a maximum and the letter Z the lower limit at which elongation occurs. It may be noted that there is a very sharp decrease in the sugar at about the region where growth is at a maximum. The very rapid use of sugar for the formation of cellulose and other structural substances and the diluting effect of the absorbed water probably account for its very low concentration in this region. FERNALD (7) has found that the freezing point depression is maximum at the tip, decreases for a distance below the tip, and then becomes somewhat irregular; it generally increases again toward the basal end of the stalk. This does not seem to be related to the sugar content, but to a certain extent parallels the total solids content. Several factors are evidently concerned.

#### TOTAL NITROGEN

The percentage of total nitrogen in successive segments along the stalk plotted against the height of the segment above the base is shown in figure 4. It is apparent that the percentage of nitrogen is high at the tip and low

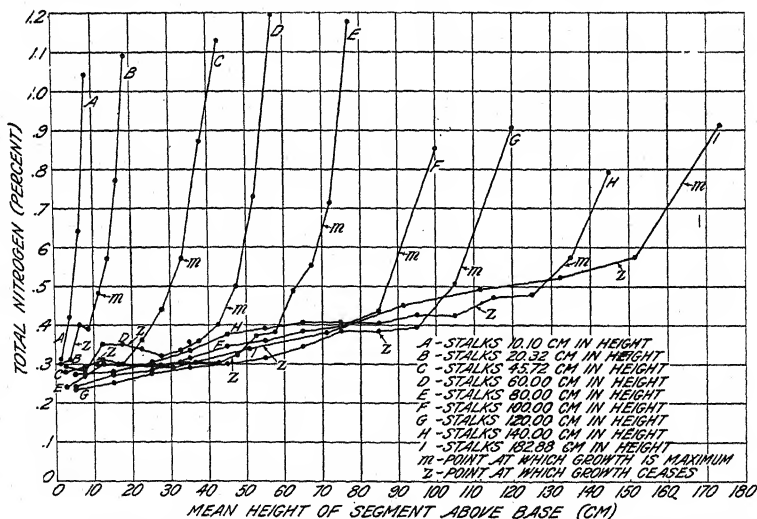


FIG. 4. Percentage of total nitrogen in asparagus in successive segments along the stalks of various heights.

in the basal portion of stalks of all heights. In young stalks 10.1 cm. tall the percentage of nitrogen increases rapidly from base to tip; the same is true for those 20.3 cm. tall to a somewhat smaller degree. In stalks 60 cm.

tall the nitrogen is fairly low for about 20 cm. above the base, then rises rapidly to a maximum at the tip. In those 100 cm. tall, it rises slowly to about 80 cm. in height and then increases rapidly to the tip. The region of maximum growth corresponds approximately to that at which the percentage of nitrogen begins its rapid rise. In other words, the points of inflection on the curves correspond very roughly to zones in which growth is at a maximum. Below these zones the curves are markedly flat. It is also evident that in the zone where growth ceases the percentage of total nitrogen has fallen to a low level. Also from the very high moisture content at this point it may be inferred that the protoplasm has become greatly vacuolated or has taken into its confines large amounts of water which lowers the total nitrogen content on the fresh weight basis.

The percentage of total nitrogen in the segments at the tip remains surprisingly near the same value in the tall as in the short stalks. The results are somewhat irregular and the values tend to decrease slightly in the taller stalks. The irregularity is probably due in part to sampling error while the tendency to decrease is probably the result of analyzing a longer segment, and of a relative decrease in nitrogen supply.

The question arises as to how the stalk maintains a high percentage of nitrogen at the tip in spite of its rapid elongation. This may be explained by the transportation of soluble nitrogen from the roots to the apical portion of the stalk before and shortly after it emerges from the soil. Up to this time increase in length has been relatively slow but increase in diameter has been comparatively rapid. By the time the stalk is 10 to 20 cm. tall, it has almost reached its full diameter at the surface of the soil and large amounts of nitrogenous substances have accumulated in the region from the apex downward for some distance.

In table III is given the fresh weight, the percentage of total nitrogen on the fresh weight basis, and the total weight of the nitrogen in 1-cm. sections of the fresh stalk at different heights. The fresh weight and chemical data were secured from different sets of stalks. For this reason the total weight of nitrogen per cm. of stalk may be subject to considerable sampling error but every effort was made to select comparable material.

It is noted in table III that the weight of the apical section decreases as the stalks become taller. In stalks 10.16, 20.32, 60, and 80 cm. in height, the weights of the tip segments were 1.21, 1.01, 0.706, and 0.563 gm. respectively. In the same way the segment immediately below the tip decreased in weight with an increase in height of the stalks and so on with the third and fourth segment below the tip.

The absolute weight of nitrogen also decreases in the tip segment as the stalk increases in height. In stalks 10.16, 60, and 80 cm. high, the tip centimeter has 0.0126, 0.011, and 0.0066 gm. of nitrogen respectively. In the

same way the segment immediately below the tip decreases in its absolute amount of nitrogen as the stalk becomes higher. In general the same ten-

TABLE III

FRESH WEIGHT AND TOTAL NITROGEN CONTENT OF 1-CM. SEGMENTS OF ASPARAGUS STALKS  
AT DIFFERENT HEIGHTS FROM THE BASE

TOTAL HEIGHT OF STALK	HEIGHT OF SEGMENT ABOVE BASE	FRESH WEIGHT OF 1-CM. SEG- MENT OF STALK	TOTAL NITROGEN FRESH WEIGHT BASIS	WEIGHT OF NITROGEN IN 1-CM. SEGMENT OF STALK
<i>cm.</i>	<i>cm.</i>	<i>gm.</i>	<i>%</i>	<i>gm.</i>
10.16	1.27	2.40	0.31	0.00744
"	3.81	2.33	0.42	0.00978
"	6.35	2.12	0.64	0.01356
"	8.89	1.21	1.04	0.01260
20.32	1.27	2.78	0.30	0.00735
"	3.81	2.52	0.31	0.00781
"	6.35	2.19	0.40	0.00876
"	8.89	2.18	0.42	0.00916
"	11.43	2.00	0.48	0.00955
"	13.97	1.75	0.57	0.00995
"	16.51	1.48	0.77	0.01140
"	19.05	1.01	1.09	0.01100
45.72	2.54	2.71	0.28	0.00760
"	7.62	2.67	0.29	0.00776
"	12.70	2.42	0.31	0.00749
"	17.78	2.53	0.30	0.00760
"	22.86	2.15	0.36	0.00775
"	27.94	1.81	0.44	0.00795
"	33.02	1.51	0.57	0.00862
"	38.10	1.16	0.87	0.01010
"	43.18	0.88	1.13	0.00991
60	2.5	2.49	0.294	0.00732
"	7.5	2.65	0.282	0.00749
"	12.5	2.53	0.301	0.00758
"	17.5	2.21	0.350	0.00773
"	22.5	2.18	0.338	0.00735
"	27.5	2.23	0.321	0.00715
"	32.5	2.03	0.335	0.00682
"	37.5	1.93	0.359	0.00692
"	42.5	1.76	0.405	0.00715
"	47.5	1.55	0.500	0.00778
"	52.5	1.19	0.739	0.00882
"	57.5	0.706	1.195	0.00840
80	2.5	2.96	0.241	0.00714
"	7.5	2.70	0.267	0.00720
"	12.5	2.33	0.310	0.00730
"	17.5	2.36	0.306	0.00722
"	22.5	2.51	0.300	0.00754
"	27.5	2.03	0.296	0.00700
"	32.5	2.17	0.304	0.00662
"	37.5	1.96	0.302	0.00593
"	42.5	1.92	0.304	0.00584

TABLE III—(Continued)

FRESH WEIGHT AND TOTAL NITROGEN CONTENT OF 1-CM. SEGMENTS OF ASPARAGUS STALKS  
AT DIFFERENT HEIGHTS FROM THE BASE

TOTAL HEIGHT OF STALK	HEIGHT OF SEGMENT ABOVE BASE	FRESH WEIGHT OF 1-CM. SEG- MENT OF STALK	TOTAL NITROGEN FRESH WEIGHT BASIS	WEIGHT OF NITROGEN IN 1-CM. SEGMENT OF STALK
<i>cm.</i>	<i>cm.</i>	<i>gm.</i>	<i>%</i>	<i>gm.</i>
"	47.5	1.77	0.324	0.00573
"	52.5	1.57	0.371	0.00585
"	57.5	1.48	0.380	0.00562
"	62.5	1.17	0.487	0.00570
"	67.5	1.19	0.553	0.00638
"	72.5	1.15	0.713	0.00695
"	77.5	0.563	1.177	0.00663
100	5	2.66	0.240	0.00638
"	15	2.62	0.270	0.00710
"	25	2.41	0.295	0.00710
"	35	2.13	0.317	0.00675
"	45	1.95	0.349	0.00667
"	55	1.66	0.360	0.00600
"	65	1.34	0.385	0.00518
"	75	1.08	0.397	0.00425
"	85	0.82	0.435	0.00357
"	95	0.50	0.853	0.00426
120	5	2.71	0.244	0.00661
"	15	2.68	0.251	0.00672
"	25	2.52	0.277	0.00694
"	35	2.23	0.295	0.00658
"	45	1.92	0.304	0.00584
"	55	1.75	0.316	0.00553
"	65	1.40	0.347	0.00486
"	75	1.16	0.387	0.00449
"	85	0.94	0.385	0.00375
"	95	0.69	0.396	0.00273
"	105	0.40	0.506	0.00202
"	115	0.30	0.905	0.00271
140	5	2.62	0.274	0.00710
"	15	2.60	0.278	0.00723
"	25	2.45	0.300	0.00735
"	35	2.20	0.337	0.00748
"	45	1.92	0.376	0.00723
"	55	1.68	0.392	0.00658
"	65	1.40	0.407	0.00570
"	75	1.19	0.406	0.00483
"	85	1.02	0.401	0.00409
"	95	0.80	0.426	0.00341
"	105	0.61	0.421	0.00257
"	115	0.44	0.470	0.00207
"	125	0.33	0.573	0.00189
"	135	0.26	0.759	0.00197

dency is evident in the third and fourth segment below the tip. A more complete picture of the nitrogen trends is shown in figure 5. The absolute



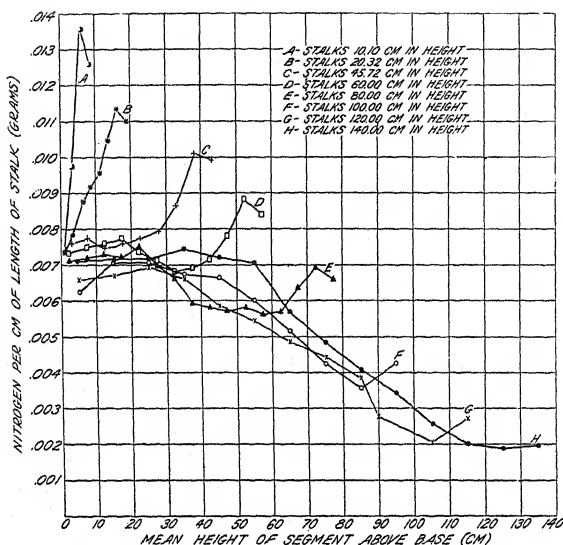


FIG. 5. The absolute amount of nitrogen per cm. length of the stalk of asparagus in successive segments from base to tip.

amount along the stalk also varies from base to tip and is greatest near the tip, particularly in stalks up to 60 cm. in height. In stalks above 80 cm. in height there is a tendency for the absolute amount to decrease along the stalk from the base to the region of the growing zone when it again increases to the tip. This is owing to the difference in the total weight of the segments of different heights and to the percentage of nitrogen present. Being a monocotyledonous plant, the diameter of the stem becomes fixed by morphological limitations a few centimeters below the tip.

It is well known that through the activity of the protoplasm or the nitrogenous substances the formation of the structural materials is effected. The cells when first formed at the apex are densely filled with protoplasm and a short distance below the tip they begin a rapid extension in size by the absorption of water which is then followed by a thickening and further differentiation of the structural elements that continues until the stem is fully mature. Thus a small amount of nitrogenous substances in the apical portion of the stalk may suffice to form a large amount of structural tissues and the reserve supply in the tip appears to be used in this way. With these facts in mind it is not surprising that the plant is able to maintain the percentage of nitrogen in the tip at a rather high value during the period of most rapid elongation.

#### TITRATABLE ACIDITY

The titratable acidity is lowest in the basal segments and highest near the tip (table II). The higher acidity near the tip may be caused by a greater

amino acid content in this region. No tests were made to determine the active acidity, or hydron concentration, but it closely parallels the titratable acidity in many plant materials. CALDWELL (4) has shown that in the growth of many young fruits the rapid increase in size and hydration of the tissues of the fruit is accompanied by an increased active acidity. The acidity was generally highest in the tip segment; but in stalks 60 and 80 cm. tall it was highest in approximately the region of maximum growth rate. More work is necessary to establish the character of the acidity gradient in the growing stalks.

#### TANNIN CONTENT

The substances which account for the values here obtained are known only in part for there is no doubt that substances other than true tannins enter into the reactions. These substances vary considerably in amount in the tissues of the stalks at different heights. They are always lowest toward the base and highest near the tip. Their distribution along the stalk is shown in figure 6. The percentage present in any region appears to be closely

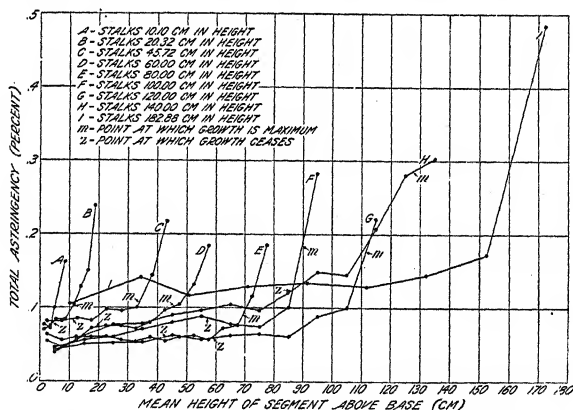


FIG. 6. The percentage of total astringency in asparagus in successive segments along the stalks of various heights.

correlated with the total nitrogen. They are high in amount during the early growth processes but decrease to a low level below the point on the stalk where growth ceases. They also appear to be higher in the green portion of the stalk than in the portion with little green color present.

#### ACID-HYDROLYZABLE POLYSACCHARIDES

According to TANRET (11) starch does not occur in the asparagus plant but inulin-like substances are present instead. It is apparent from table II that the acid-hydrolyzable polysaccharides are generally very low. Although the values appear somewhat erratic there is a tendency for these

substances to be lowest in the region between the points of maximum growth and cessation of growth. Hydrolysis with hydrochloric acid probably destroys some of the levulose which makes the results somewhat irregular.

### Summary

1. Using field grown material, the rate of elongation of the stem of asparagus in different zones along the stem was determined for plants of widely different heights at temperatures between 65 and 70° F. and the results plotted. The rates of elongation vary in a characteristic manner with each height of stalk. The zone of maximum growth changes its position relative to the tip with each height of stalk; the length of the growing region and the rate of growth in this region varies with each height of the plant. The rates of elongation in the zones of maximum growth are nearly constant over a rather wide range of heights. There is a tendency for this maximum value to decrease somewhat with increase in height for stalks from 15 to 195 cm. high. The difference in the total elongation per 24 hours among stalks of different heights is caused principally by the difference in the length of the growing region and not by differences in the maximum rate of growth per unit length of growing zone.

2. Analyses made of segments of the stalk at different heights show that the total solids vary greatly at different points along the stalk. The total solids were lowest in a zone several centimeters below the tip, varying somewhat with the total height of the stalk. In stalks of intermediate height the curves representing the relationship are distinctly U-shaped, the lowest total solids content being found in the middle portion.

It appears that the lowest solids or the highest moisture is in a region between that at which growth is at a maximum and the point where it ceases. It is concluded that a certain amount of thickening of the cell walls occurs before the growth finally ceases.

3. Total sugars are highest at the base and decrease rapidly to a low value near the tip. They decrease very rapidly in the growth zone and above the point of maximum rate of elongation they decrease to a very low value. This may be due to the rapid use of the sugars in cellulose formation. The amount at any point is determined partly by the rate at which they are used, and partly by the rapidity with which they may be transported from the source of supply to the region where they are being used.

4. Total nitrogen is lowest in the basal portion of the stalk but increases rapidly in amount in regions near the tip. Total nitrogen or the density of the protoplasm constantly changes as growth development occurs in the growing region. The elongation of the stalk is accompanied by hydration of the cells which apparently continues until the cell walls become sufficiently

rigid to check the process. At the point where growth ceases the total nitrogen has decreased to a low value. In the region immediately above and below the point where growth ceases there is a rapid addition to the stalk of cellulose and other structural materials. In spite of these processes total nitrogen remains high at the extreme tip of the stalk. The total amount per unit length of the stalk, however, in the region near the tip does decrease as growth proceeds.

5. Changes in total astringency are very closely correlated with changes in the total nitrogen. The total astringency is lowest towards the base and highest at the tip.

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# FOLIAR DIAGNOSIS: PHYSIOLOGICAL BALANCE BETWEEN THE BASES LIME, MAGNESIA, AND POTASH<sup>1</sup>

WALTER THOMAS AND WARREN B. MACK

(WITH FOUR FIGURES)

## Introduction

One of the functions of calcium is to act as a base in neutralizing the acids of the tissues (5). Although other bases take part in the structure and functioning of plants, in terrestrial plants two bases, magnesia and potash, are of such importance as always to be employed in studies of plant nutrition. In certain respects each of these base-forming elements appears to fill a specific rôle; but considered as bases, functioning solely in their rôle of neutralizing the acids of the tissues, CaO, MgO, and K<sub>2</sub>O are able chemically to replace one another equivalent for equivalent. If the plant selects one rather than another, it is the result of its normal or natural power to regulate the absorption of solutes. The object of this investigation is to examine the nature of this regulation.

In a cosmos regulated by immutable laws it would be logical to expect that the absorption of mineral elements would conform to a definite system, as evidence of which may be cited the fact that when growth is optimum the absorption of nitrogen and phosphoric acid is a linear function with time (2, 6).

In the studies on the method of leaf diagnosis thus far carried out (7, 8, 9, 10), calcium and magnesium were not determined because of the use (on the potato) of calcium-containing sprays. The fact was emphasized, however, that the basic assumption in fertilizer field plot experiments is that the factors other than the ones being investigated are nearly equal for the plots to be compared (7). A rigid examination of the effect of lime should accordingly comprise plots with and without lime for all treatments investigated.

In our investigations of the leaf, the determination of calcium and magnesium was first made on *Zea mays* growing on tier 1 of the Jordan fertility plots. In the following year the inclusion of Ca and Mg in the foliar diagnosis of the potato was made possible by omitting calcium-containing sprays.

## Foliar diagnosis of maize

### METHODS

PLOTS EXAMINED WITH THEIR TREATMENT.—The general plan of the Jordan Fertility experiments has been published by NOLL (2). The soil

<sup>1</sup> Authorized for publication as paper no. 862 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

is formed by the disintegration of limestone rocks. In some of the plots, notably nos. 1-5, the parent rock is near the surface. Only two of the plots on tier 1 on which the present experiments were conducted had received lime; the other plots had not received any lime since 1881 (11, 12).

The results reported in this paper are those from plants growing on plots receiving single element treatments, combination of two elements, and also of all three elements. In addition a plot receiving manure and lime, and another lime alone and also an unfertilized (nothing) plot were included. The plots with treatments and yields are listed in table I.

TABLE I  
PLOT TREATMENTS WITH YIELDS OF MAIZE

PLOT	SYMBOL	TREATMENT	YIELDS OF GRAIN PER PLOT
			<i>lb.</i>
1 .....	Nothing	No lime	165.4
2 .....	N	No lime, N as dried blood	265.6
3 .....	P	No lime, P as superphosphate	371.2
4 .....	K	No lime, K as muriate	298.3
5 .....	NP	No lime, N as dried blood P as superphosphate	376.7
6 .....	NK	No lime, N as dried blood K as muriate	263.5
7 .....	PK	No lime, P as superphosphate K as muriate	497.4
9 .....	NPK	No lime, N as dried blood P as superphosphate K as muriate	520.2
26 .....	NPK	No lime, N as sodium nitrate P as superphosphate K as muriate	705.5
22 .....	Manure + lime }	6 tons of manure	770.8
23 .....		2 tons of lime every 4 years	452.2
		2 tons of lime every 4 years	

LEAF SAMPLING.—The third leaf from the base was sampled in the manner previously described (7, 9) at four periods—*viz.*, July 6, July 21, August 8, and August 25. On two plots, check and manure (the lowest and highest yielding plots on this tier) samples were taken also on June 16.

#### PRESENTATION OF RESULTS

DERIVATION OF THE Ca-Mg-K-UNIT.—The results of the analysis for CaO, MgO, and K<sub>2</sub>O in the third leaf are shown in table II (columns 4, 5, and 6). Inasmuch as the chemical reactions within the leaf are under consideration, these percentages of CaO, MgO, and K<sub>2</sub>O in the dried foliage are converted into milligram equivalents (*E<sub>x</sub>*, *E<sub>y</sub>*, *E<sub>z</sub>*, columns 7, 8, and 9). The last three columns show the values of the composite *Ca-Mg-K-unit*, which

TABLE II

PERCENTAGES OF  $\text{CaO}$ ,  $\text{MgO}$  AND  $\text{K}_2\text{O}$  IN THE DRIED FOLIAGE OF MAIZE, THEIR MILLIGRAM EQUIVALENT VALUES, AND THE COMPOSITION OF THE  $\text{Ca-Mg-K-unit}$

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE			MILLIGRAM EQUIVALENT			COMPOSITION OF THE $\text{Ca-Mg-K-unit}$		
			$\text{CaO}$ ( $\text{M}_x$ )	$\text{MgO}$ ( $\text{M}_y$ )	$\text{K}_2\text{O}$ ( $\text{M}_z$ )	$\text{CaO}$ ( $\text{E}_x$ )	$\text{MgO}$ ( $\text{E}_y$ )	$\text{K}_2\text{O}$ ( $\text{E}_z$ )	X $\left(100 \cdot \frac{\text{E}_x}{S}\right)$	Y $\left(100 \cdot \frac{\text{E}_y}{S}\right)$	Z $\left(100 \cdot \frac{\text{E}_z}{S}\right)$
			%	%	%	mg. eq.	mg. eq.	mg. eq.			
June 16 .....	1	Check	2.510	0.910	2.500	89.607	45.318	53.250	47.300	23.090	29.610
July 6 .....			2.308	0.934	2.387	82.396	45.513	50.843	46.095	25.461	28.443
July 21 .....			2.087	0.966	2.515	74.506	48.107	53.570	42.289	27.305	30.406
Aug. 8 .....			1.605	1.039	1.841	57.299	51.742	39.213	38.649	34.901	26.450
Aug. 25 .....	2	N	1.999	1.216	1.608	71.364	60.557	34.250	52.946	36.443	20.611
July 6 .....			2.058	1.177	2.248	73.471	58.615	47.882	40.824	32.570	26.606
July 21 .....			2.435	1.448	2.104	86.929	72.110	44.815	42.643	35.373	21.984
Aug. 8 .....			2.328	1.376	1.453	83.110	68.525	30.949	45.519	37.531	16.950
Aug. 25 .....	3	P	2.222	1.159	1.481	79.325	57.718	31.545	47.053	34.236	18.711
July 6 .....			3.131	1.901	1.019	111.777	94.670	21.705	48.992	41.494	9.513
July 21 .....			3.744	2.462	0.756	133.611	122.608	16.103	49.073	45.015	5.912
Aug. 8 .....			3.348	1.847	0.794	119.524	91.981	16.912	52.327	40.269	7.404
Aug. 25 .....	4	K	2.937	1.792	0.911	104.851	89.242	19.404	49.111	41.800	9.089
July 6 .....			1.690	0.815	3.984	60.333	40.587	84.859	32.476	21.847	45.677
July 21 .....			1.887	1.112	4.000	67.366	55.378	85.200	32.396	26.631	40.973
Aug. 8 .....			1.689	0.905	3.901	60.297	45.069	83.091	31.995	23.915	44.090
Aug. 25 .....	5	NP	1.491	0.742	2.480	53.229	36.952	52.824	37.222	25.840	36.938
July 6 .....			3.410	2.114	0.724	121.737	105.277	15.421	50.214	43.424	6.362
July 21 .....			3.322	2.516	0.813	118.595	125.296	17.317	45.403	47.967	6.630
Aug. 8 .....			3.322	2.077	0.813	118.595	100.944	17.317	50.072	42.619	7.309
Aug. 25 .....	6	NK	2.984	2.281	0.775	106.528	113.593	16.507	45.019	48.004	6.976
July 6 .....			1.543	0.634	4.031	55.085	31.573	85.860	31.930	18.301	49.769
July 21 .....			1.510	0.863	4.209	53.907	41.483	89.652	29.132	22.418	48.450
Aug. 8 .....			1.628	0.905	3.081	58.120	45.069	65.625	34.328	26.697	38.874
Aug. 25 .....			1.309	0.525	2.763	46.731	26.145	58.852	35.475	19.848	44.677



TABLE II—(Continued.)

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE				MILLIGRAM EQUIVALENT				COMPOSITION OF THE Ca-Mg-K-UNIT		
			CaO (M <sub>x</sub> )	MgO (M <sub>y</sub> )	K <sub>2</sub> O (M <sub>z</sub> )	%	CaO (E <sub>x</sub> )	MgO (E <sub>y</sub> )	K <sub>2</sub> O (E <sub>z</sub> )	mg. eq.	X $\left(100 \cdot \frac{E_x}{S}\right)$	Y $\left(100 \cdot \frac{E_y}{S}\right)$	Z $\left(100 \cdot \frac{E_z}{S}\right)$
July 6 .....	7	PK	1.902	0.605	3.565	%	67.901	30.129	75.936	mg. eq.	39.032	17.319	43.649
July 21 .....			2.221	0.899	3.508	%	79.290	41.284	74.720		40.600	21.139	38.260
Aug. 8 .....			2.283	0.888	2.887	%	81.503	34.262	61.493		45.980	19.329	34.691
Aug. 25 .....			1.917	0.507	2.655	%	68.437	25.249	56.551		45.553	16.806	37.641
July 6 .....	9	NPK (N as dried blood)	1.720	0.606	3.674	%	61.404	30.179	78.256		36.154	17.769	46.077
July 21 .....			1.887	0.707	3.766	%	67.366	35.209	80.216		36.854	19.262	43.884
Aug. 8 .....			1.842	0.362	2.992	%	65.759	18.028	63.730		44.577	12.221	43.202
Aug. 25 .....			1.705	0.271	2.879	%	60.869	13.496	61.323		44.860	9.946	45.194
July 6 .....	26	NPK (N as sodium nitrate)	1.933	0.471	3.837	%	69.008	23.456	81.728		39.616	13.466	46.918
July 21 .....			2.313	0.670	3.643	%	82.574	33.366	77.596		42.666	17.240	40.090
Aug. 8 .....			2.237	0.453	2.946	%	79.861	22.559	62.750		48.351	13.658	37.991
Aug. 25 .....			2.207	0.525	2.267	%	78.790	26.145	43.287		51.422	17.063	31.514
June 16 .....	22	manure + lime	2.410	0.540	2.710	%	86.037	26.892	57.723		50.245	15.600	34.155
July 6 .....			2.793	0.630	2.740	%	99.710	31.374	58.362		52.632	16.561	30.807
July 21 .....			3.160	0.717	2.752	%	112.812	35.707	58.618		54.463	17.238	28.299
Aug. 8 .....			3.381	0.760	2.092	%	120.702	37.848	44.560		59.427	18.634	21.939
Aug. 25 .....	23	lime	3.514	0.851	1.890	%	125.449	42.600	40.400		60.395	20.105	19.500
July 6 .....			4.144	1.376	0.872	%	147.941	68.525	18.574		62.943	29.155	7.902
July 21 .....			4.233	1.231	0.910	%	151.118	61.304	19.383		65.192	26.446	8.362
Aug. 8 .....			3.851	1.122	0.910	%	137.481	55.876	19.383		64.624	26.265	9.111
Aug. 25 .....			3.983	1.014	0.891	%	142.193	50.497	18.978		67.177	23.857	8.966

represents the equilibrium between  $\text{CaO-MgO-K}_2\text{O}$  at the moment of sampling, and is derived by determining the proportion each of the milligram equivalent values for  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$  bears to the total. Hence  $\frac{E_x}{S} + \frac{E_y}{S} + \frac{E_z}{S} = 1$ . To avoid fractions, the magnitudes so obtained are multiplied by 100; therefore

$\left(100 \frac{E_x}{S}\right) + \left(100 \frac{E_y}{S}\right) + \left(100 \frac{E_z}{S}\right) = 100$ , or  $X + Y + Z = 100$ . The values of  $X + Y + Z$  for any given sample are the composite *Ca-Mg-K-unit* as defined above.

#### DISCUSSION AND INTERPRETATION OF RESULTS

RELATION BETWEEN  $\text{CaO-MgO-K}_2\text{O}$  UNDER OPTIMUM GROWTH CONDITIONS IS A LINEAR FUNCTION.—The *Ca-Mg-K-units* given in the last three columns of table II are plotted in trilinear coordinates in figure 1. The numerals 0, 1, 2, 3, 4 indicate the dates of sampling, June 16 ( $t=0$ ), July 6, July 21, August 8, August 25, respectively; on graphs without these numerals, the arrow with the treatment symbol indicates the first date (July 6), and others follow in order. As already stated, samples on June 16 ( $t=0$ ) were taken only from plants growing on the check and manure plots.

The broken line  $ee'$  joins the coordinate points for samples taken July 6

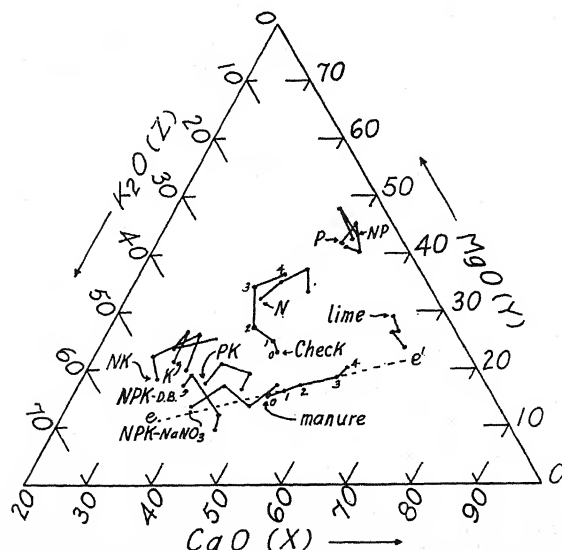


FIG. 1. Deviations from the optimum physiological balance (the line  $ee'$ ) between  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$  resulting from single "element," combination of two "elements," and a complete fertilizer carrying nitrogen in the two forms dried blood (D.B.) and sodium nitrate ( $\text{NaNO}_3$ ) (maize experiments).

and August 8 from the maximum yielding plot (manure + lime). Equations of this line may be expressed in two ways, *viz.*,  $ax + by + cz = 0$ , where  $x$ ,  $y$ , and  $z$  are the perpendicular distances drawn from the coordinate points to the sides of the triangle, and  $a$ ,  $b$ , and  $c$  are constants; or in the standard form of the equation of a straight line  $y = mx + b$ .

Because of the fact that a line drawn from any point within an equilateral triangle perpendicular to one of the sides bears a constant relation to a line drawn from the same point to the same side, but parallel to one of the other two sides, it may be shown that the ordinates  $X$ ,  $Y$ , and  $Z$  representing the *Ca-Mg-K-unit* may be substituted for  $x$ ,  $y$ , and  $z$  of the first equation shown above. The equation of the line  $ee'$  in trilinear coordinates, therefore, may be written in the form  $aX + bY + cZ = 0$ , or  $X + \left(\frac{b}{a}\right)Y + \left(\frac{c}{a}\right)Z = 0$ , and with the equation in this form it is possible to determine the values of  $\left(\frac{b}{a}\right)$  and  $\left(\frac{c}{a}\right)$  from the coordinates of any two points on the straight line. If the *Ca-Mg-K-units* for manure on July 6 (52.632: 16.561: 30.807) and on August 9 (59.427: 18.634: 21.939) are taken as the coordinates of these two points, the values of  $\left(\frac{b}{a}\right)$  and  $\left(\frac{c}{a}\right)$  are found to be  $-3.2079$  and  $0.0161$ , respectively. The equation of the line  $ee'$  in trilinear coordinates, therefore, is  $X - 3.2079Y + 0.161Z = 0$ .

With observed values of  $MgO$  and  $K_2O$  at the different dates of sampling as  $Y$  and  $Z$ , the values of  $CaO$  at the respective dates may be calculated from the foregoing equation. The observed ( $X$ ) and calculated ( $X_1$ ) values thus computed and the ratio of the difference between them to the observed values  $\left(\frac{X-X_1}{X}\right)$  are given in table III.

The simplest form of equation, and the one easiest to interpret physiolog-

TABLE III

EXPERIMENTAL VALUES AND VALUES CALCULATED FROM THE EQUATION IN TRILINEAR COORDINATES REPRESENTING THE EQUILIBRIUM AMONG  $CaO$ ,  $MgO$ , AND  $K_2O$  AT FIVE SUCCESSIVE DATES OF SAMPLING FOR PLANTS OF *Zea mays* GROWING ON THE MANURE PLOT

DATES OF SAMPLING	OBSERVED VALUES OF $CaO$ ( $X$ )	CALCULATED VALUES OF $CaO$ ( $X_1$ )	DIFFERENCE, $\frac{X - X_1}{X}$
June 16 .....	50.245	49.493	0.015
July 6 .....	52.632	52.632	0.0
July 21 .....	54.463	54.842	-0.007
Aug. 8 .....	59.427	59.427	0.0
Aug. 25 .....	60.395	64.182	-0.062

ically, is the linear one  $Y = mX + b$ , where  $X$  is the abscissa ( $\text{CaO}$ ), and  $Y$  the ordinate ( $\text{MgO}$ ). In this form the equation of the line  $ee'$  is  $Y = 0.305 X + 0.050$ . It is seen that with the exception of the values for the last sampling date, when chlorophyll degeneration had already begun, all the other values for this manure + lime plot (no. 22) lie sensibly on this line  $ee'$ . Since  $X + Y + Z = 100$ , and since  $Y = mX + b$ , it follows also that any two of these variables  $X$ ,  $Y$ ,  $Z$  are related by a linear equation.

The equation connecting the values for  $\text{CaO}$  ( $X$ ) and  $\text{K}_2\text{O}$  ( $Z$ ) is  $Z = -1.305X + 99.490$  and that relating the values for  $\text{MgO}$  ( $Y$ ) and  $\text{K}_2\text{O}$  ( $Z$ ) is  $Z = -0.233 Y + 23.760$ .

DEVIATION OF EXPERIMENTAL FROM CALCULATED VALUES.—The deviation of the experimental values from the values calculated from these equations is given in table IV. The values given in the last column represent the relative deviation of the milligram equivalent values, which are in the same proportion to each other as the values  $X$ ,  $Y$ , and  $Z$  of the *Ca-Mg-K-units*.

It is seen that with the exception of the last sampling date the experimental values deviate very little from the calculated values.

TABLE IV

EXPERIMENTAL VALUES AND VALUES CALCULATED FROM THE THREE EQUATIONS REPRESENTING THE EQUILIBRIUM BETWEEN  $\text{CaO-MgO}$ ,  $\text{CaO-K}_2\text{O}$ , AND  $\text{K}_2\text{O-MgO}$   
AT FIVE SUCCESSIVE DATES OF SAMPLING FOR PLANTS OF *Zea*  
*mays* GROWING ON THE MANURE PLOTS

DATES OF SAMPLING	EXPERIMENTAL VALUES		CALCULATED VALUES	RELATIVE DEVIATION
	MgO ( $Y$ )	CaO ( $X$ )	CaO ( $X$ )	$\frac{X - X_1}{X}$
June 16 .....	15.600	50.245	49.493	+ 0.014
July 6 .....	16.561	52.632	52.632	0.0
July 21 .....	17.238	54.463	54.865	- 0.008
Aug. 8 .....	18.634	59.427	59.427	0.0
Aug. 25 .....	20.105	60.395	64.262	- 0.063
	$\text{K}_2\text{O}$ ( $Z$ )	CaO ( $X_2$ )	CaO ( $X_1$ )	$\frac{X - X_2}{X}$
June 16 .....	34.155	50.245	50.065	+ 0.003
July 6 .....	30.807	52.632	52.632	0.0
July 21 .....	28.299	54.463	54.865	- 0.004
Aug. 8 .....	21.939	59.427	59.427	0.0
Aug. 25 .....	19.500	60.395	61.226	- 0.013
	MgO ( $Y$ )	$\text{K}_2\text{O}$ ( $Z$ )	$\text{K}_2\text{O}$ ( $Z_1$ )	$\frac{Z - Z_1}{Z}$
June 16 .....	15.600	34.155	35.021	- 0.025
July 6 .....	16.561	30.807	30.807	0.0
July 21 .....	17.238	28.299	28.000	+ 0.010
Aug. 8 .....	18.634	21.939	21.939	0.0
Aug. 25 .....	20.105	19.500	15.691	+ 0.190

SIGNIFICANCE OF THE EQUATIONS.—These equations show that as the values for CaO increase with maturity the values for MgO also become larger and by regular increments. On the other hand as the values for CaO increase, those for  $K_2O$  decrease regularly. Similarly, as the values for  $K_2O$  decrease those for MgO increase regularly also.

These relationships are of interest in relation to their bearing on observations by earlier workers which hitherto were incompletely understood. LOEW (4) concluded that plants require a definite CaO/MgO ratio in their medium, and that this requirement varied with the plant species. This hypothesis was succeeded by EHRENBURG's so-called "lime-potash law" (1) according to which lime reduces the amount of potassium taken up by plants. Later WIEGNER and MÜLLER (13) were able to show that the concentration of potassium in the aqueous phase of the soil is proportional to the sum of the potassium and calcium dissolved from the absorbing soil complex.

Although the amounts of nutrient and other materials in the plant are determined by the quantities available, the absorption of elements is not in the proportion in which they exist in the soil, but in proportions modified by certain characteristics of the plant (5). The equilibrium between CaO-MgO, between CaO- $K_2O$ , and between MgO- $K_2O$  indicated by the above equations shows the relative proportion in which they are present in the leaf during the growth cycle of *Zea mays*. When one or another of the elements is not present in sufficient amounts in the soil, the plant responds by a relatively excess absorption of one of the others, resulting in a disequilibrium which is reflected in reduced yields.

The seat of the inhibition of the absorption of potassium by calcium then lies in the soil and not within the plant.

DEVIATIONS FROM EQUILIBRIUM CONDITIONS WITH CHARACTERISTICS OF THE GRAPHS OF THE DIFFERENT TREATMENTS.—The deviations from equilibrium conditions resulting from the other treatments are shown in figure 1. The graphs are compared with that of the highest yielding treatment (manure + lime, plot 22), represented by the line  $ee'$ .

The characteristics of these graphs: *Check* (no. 1) [no fertilizer, no lime].—Relative to the treatment highest yielding, manure + lime (plot 22) the original unfertilized soil contains (with respect to *Zea mays*) great excess (relative) of MgO, deficiency of CaO, and sufficient  $K_2O$ .

*Nitrogen* (no. 2) [no lime, no phosphoric acid, and no potash].—Deficiency (relative) of CaO, great excess of MgO, and slight deficiency of  $K_2O$ .

*Phosphorus* (no. 3) [no lime, no nitrogen, no potash].—The displacement towards the right side of the triangle and high up towards the summit indicates great excess (relative) of MgO, slight deficiency of CaO, and great deficiency of  $K_2O$ .

*Potash* (no. 4) [no lime, no nitrogen, no phosphorus].—The displace-

ment is well over to the left side of the triangle indicating great deficiency (relative) of CaO and excess of  $K_2O$  and also of MgO.

*Nitrogen and phosphorus (no. 5)* [no lime, no potash].—Slight deficiency (relative) of CaO becoming greater with maturity, great excess of MgO, and great deficiency of  $K_2O$ .

*Nitrogen and potash (no. 6)* [no lime, no phosphorus].—Great deficiency (relative) of CaO, and excess of  $K_2O$  and also of MgO.

*Phosphorus + potash (no. 7)* [no lime, no nitrogen].—Relative deficiency of CaO, and excess of  $K_2O$  and of MgO.

*Nitrogen + phosphorus + potash (no. 9)* [no lime, nitrogen as dried blood].—Deficiency of CaO, excess of  $K_2O$ , and variable MgO, which is too high at first and with advancing age of the leaf becomes too low.

*Nitrogen + phosphorus + potash (no. 26)* [no lime, nitrogen as sodium nitrate].—In comparison with no. 9 (N as dried blood), sodium nitrate has increased the CaO and reduced the  $K_2O$ , but has changed the MgO but little.

*Lime (no. 23)* [no nitrogen, no phosphoric acid, and no potash].—Great deficiency (relative) of potash, great excess of CaO, and slight excess of MgO.

In [P], [NP], and [lime] particularly, and to a lesser extent in [check] and [N], then, there is relative insufficiency of  $K_2O$ . In these cases the demand of meristematic tissues results in export of potassium, which is normally necessary, from the leaf and accumulation therein of an abnormal proportion of CaO and MgO in the place of the  $K_2O$ . The leaf then is exhausted cell by cell until finally it dies. This degeneracy of the leaf was evident towards the latter part of July in [P], [NP], [check], and [N].

### Foliar diagnosis of the potato

#### METHODS

The inclusion of calcium and magnesium in the investigations on the leaf diagnosis of the potato was first carried out in 1937, on section D of the vegetable fertility plots (7, 9, 10). These plots had been limed with approximately four-fifths of the quantity of ground high-calcium limestone calculated to meet the theoretical lime requirement as determined by a modified VEITCH method. The plants were sprayed weekly with cuprous oxide suspended in water by means of a neutral wetting agent, at the rate of two pounds of cuprous oxide to 100 gallons of water. Considerable damage was done to the plants by a severe attack of green apple leafhoppers, which were not controlled by the addition of an aliphatic thiocyanate insecticide (Lethane 440) which was included regularly in the cuprous oxide spray according to manufacturers' directions after the leafhoppers appeared.

The attack was of such severity that by August 20th no living leaves older than the 8th from the bottom could be found on plants on the manure plots.

On other plots damage was evident, but the lower leaves had not fallen off to such an extent as they had on the manure plots.

THE PLOTS EXAMINED, WITH THEIR TREATMENTS.—The plots with treatment examined in this paper are given in table V.

TABLE V  
PLOTS STUDIED, WITH TREATMENTS AND YIELDS OF TUBERS

TIER	PLOT	TREATMENT	SYMBOL	YIELDS PER PLOT
		<i>lb.</i>		<i>lb.</i>
1	2	3.75 lb. NaNO <sub>3</sub>	N	36.0
1	3	6.25 superphosphate (16%)	P	43.6
1	4	1.60 muriate (50%)	K	49.7
1	6	3.75 NaNO <sub>3</sub> and 6.25 superphosphate	NP	50.3
1	7	3.75 NaNO <sub>3</sub> and 1.60 muriate	NK	48.1
1	8	6.25 superphosphate and 1.60 muriate	PK	76.6
1	10	3.75 NaNO <sub>3</sub> and 6.25 superphosphate and 1.60 muriate	NPK	105.0
2	4	5.63 NaNO <sub>3</sub> and 6.25 superphosphate and 1.60 muriate	(1.5N)PK	67.0
2	8	3.75 NaNO <sub>3</sub> and 9.38 superphosphate and 1.60 muriate	N(1.5P)K	84.5
2	12	3.75 NaNO <sub>3</sub> and 6.25 superphosphate and 2.40 muriate	NP(1.5K)	90.1
2	15	5.63 NaNO <sub>3</sub> and 9.38 superphosphate and 2.40 muriate	1.5(NPK)	74.8
1	15	600 Manure	Manure	124.0

LEAF SAMPLING.—The fourth leaf from the base was sampled on July 2, July 16, August 5, in the manner previously recorded (7). Samples were taken also on August 20, but at this date leaves varying from the 6th to the 8th were the oldest available.

#### PRESENTATION OF RESULTS

Table VI shows the quantities of CaO, MgO, and K<sub>2</sub>O in the dried foliage expressed as (1) percentages, (2) milligram equivalents, and (3) the *Ca-Mg-K-unit*.

#### INTERPRETATION AND DISCUSSION OF RESULTS

CONSEQUENCES OF THE ATTACK BY LEAF HOPPERS.—In some plots (especially the manure plots) the first 8 leaves from the base were dead by August 20, and it was not possible to secure healthy leaves lower than the

TABLE VI

PERCENTAGES OF CaO, MgO, AND K<sub>2</sub>O IN THE DRIED FOLIAGE FROM POTATO PLANTS TOGETHER WITH THEIR MILLIGRAM EQUIVALENT VALUES AND THE COMPOSITION OF THE *Ca-Mg-K-unit*

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE			MILLIGRAM EQUIVALENT			COMPOSITION OF THE <i>Ca-Mg-K-unit</i>		
			CaO (M <sub>x</sub> )	MgO (M <sub>y</sub> )	K <sub>2</sub> O (M <sub>z</sub> )	CaO (E <sub>x</sub> )	MgO (E <sub>y</sub> )	K <sub>2</sub> O (E <sub>z</sub> )	X (100 $\frac{E_x}{S}$ )	Y (100 $\frac{E_y}{S}$ )	Z (100 $\frac{E_z}{S}$ )
			%	%	%	mg. eq.	mg. eq.	mg. eq.			
July 2 ..	1-2	N	2.960	1.249	3.353	105.672	62.150	71.419	44.17	25.97	29.85
July 16 ..			3.626	1.521	3.364	129.448	75.746	71.653	46.75	27.36	25.88
Aug. 5 ..			4.536	1.839	3.353	160.935	91.582	71.419	49.68	28.27	22.04
Aug. 20 ..	1-3	P	4.032	1.810	2.209	143.942	90.138	47.052	51.20	32.06	16.73
July 2 ..			4.225	1.014	3.023	150.832	50.497	64.390	56.76	19.00	24.23
July 16 ..			5.320	1.112	2.240	189.924	55.377	47.712	64.81	18.89	16.29
Aug. 5 ..	1-4	K	7.000	1.940	1.628	249.900	96.612	34.676	65.56	25.34	9.09
Aug. 20 ..			5.570	1.860	1.465	198.849	91.827	31.504	61.77	28.22	10.00
July 2 ..			2.360	0.652	5.562	84.252	32.469	118.470	35.82	13.80	50.37
July 16 ..	1-6	NP	2.772	0.669	5.833	98.960	33.316	124.244	38.57	12.98	48.44
Aug. 5 ..			3.752	0.744	5.232	113.946	36.951	109.311	43.79	14.20	42.00
Aug. 20 ..			3.710	0.706	4.624	132.447	35.158	98.491	49.77	13.21	37.01
July 2 ..	1-7	NK	4.490	1.187	2.848	160.029	59.112	60.662	57.22	21.12	21.65
July 16 ..			5.796	1.593	1.918	206.917	79.331	40.853	63.25	24.25	12.49
Aug. 5 ..			5.726	1.738	1.508	204.418	86.552	32.120	63.27	26.78	9.94
Aug. 20 ..	1-8	PK	4.676	1.955	1.124	166.333	97.359	23.941	57.91	33.77	8.31
July 2 ..			2.630	0.985	6.345	93.891	49.053	118.875	35.86	18.73	45.40
July 16 ..			2.940	1.086	5.891	104.958	54.082	125.073	36.96	19.03	44.02
Aug. 5 ..	1-8	PK	3.696	1.303	5.348	131.947	60.617	107.330	43.99	20.21	35.79
Aug. 20 ..			3.892	1.285	4.922	138.944	63.993	95.764	46.51	21.42	32.06
July 2 ..			3.400	0.579	5.213	121.380	28.834	121.367	44.69	10.61	44.69
July 16 ..	1-8	PK	4.210	0.669	5.058	150.297	33.316	117.235	49.95	11.11	38.93
Aug. 5 ..			5.016	0.687	4.554	179.071	34.212	107.330	55.85	10.67	33.47
Aug. 20 ..			4.860	0.669	4.263	173.502	33.316	70.566	62.54	12.01	25.44



TABLE VI—(Continued)

DATE OF SAMPLING	PLOT	TREATMENT	PERCENTAGE IN DRIED FOLIAGE				MILLIGRAM EQUIVALENT				COMPOSITION OF THE Ca-Mg-K-UNIT			
			CaO (M <sub>x</sub> )	MgO (M <sub>y</sub> )	K <sub>2</sub> O (M <sub>z</sub> )		CaO (E <sub>x</sub> )	MgO (E <sub>y</sub> )	K <sub>2</sub> O (E <sub>z</sub> )		X $\left(\frac{E_x}{S}\right)$	Y $\left(\frac{E_y}{S}\right)$	Z $\left(\frac{E_z}{S}\right)$	
			%	%	%	%	mg. eq.	mg. eq.	mg. eq.	mg. eq.				
July 2 ...	1-10	NPK	4.032	0.677	6.589		143.942	33.714	127.139		47.22	11.06	41.71	
July 16 ...			5.586	0.869	5.794		199.420	43.276	123.412		54.47	11.87	33.65	
Aug. 5 ...			5.922	1.028	4.360		211.415	51.194	98.661		58.51	14.16	27.32	
Aug. 20 ...			5.530	1.129	3.871		197.421	56.224	82.963		58.65	16.70	24.64	
July 2 ...	2-4	(1.5N)PK	3.320	0.641	6.527		118.522	31.922	124.264		43.14	11.62	45.23	
July 16 ...			4.382	0.779	6.124		156.437	38.794	137.044		47.08	11.67	41.24	
Aug. 5 ...			4.858	0.807	5.620		173.430	40.188	114.743		52.81	12.23	34.96	
Aug. 20 ...			4.760	0.887	4.573		169.932	44.172	103.198		53.53	13.92	32.54	
July 2 ...	2-8	N (1.5P)K	3.724	0.753	5.457		132.946	37.499	137.385		43.18	12.18	44.63	
July 16 ...			4.956	0.916	4.767		176.693	45.616	128.375		50.38	13.00	36.61	
Aug. 5 ...			5.530	1.086	5.271		197.421	54.082	96.169		56.84	15.55	27.60	
Aug. 20 ...			5.640	1.122	4.205		201.348	55.875	73.463		60.88	16.89	22.22	
July 2 ...	2-12	NP (1.5K)	3.360	0.554	7.085		119.952	27.589	145.308		40.96	9.42	49.61	
July 16 ...			4.354	0.651	6.725		155.138	32.419	146.544		46.43	9.70	43.86	
Aug. 5 ...			5.152	0.851	4.418		183.926	42.379	127.949		51.93	11.97	36.09	
Aug. 20 ...			4.620	0.896	5.019		164.934	43.276	106.073		52.48	13.77	33.74	
July 2 ...	2-15	1.5(NPK)	3.556	0.641	6.201		126.949	31.922	146.373		41.58	10.46	47.95	
July 16 ...			4.396	0.709	5.930		156.937	35.308	140.345		40.71	10.61	48.67	
Aug. 5 ...			4.718	0.832	5.290		168.432	41.433	118.044		51.36	12.63	36.00	
Aug. 20 ...			4.900	0.807	4.225		174.930	40.188	103.177		54.96	12.62	32.41	
July 2 ...	1-15	Manure	3.360	1.133	6.066		119.952	56.423	134.552		38.58	18.14	43.27	
July 16 ...			4.396	1.303	5.839		156.937	64.889	119.706		45.95	18.99	35.05	
Aug. 5 ...			5.300	1.219	5.020		189.210	60.678	107.707		53.01	17.07	30.00	
Aug. 20 ...			3.976	1.104	5.194		141.943	54.979	110.732		46.13	17.87	35.99	

sixth from the base on any of the plots at this date. Inasmuch as the basis of sampling for leaf diagnosis lies in the fact that leaves from morphologically homologous positions on the stalk are the seat of the same physiological process, leaves taken at the last sampling (August 20) are considered as being in the same physiological conditions as the fourth leaf at an earlier date. This assumption, however, will not prevent comparison being made with the maximum yielding plot (manure, no. 15).

EQUATION OF EQUILIBRIUM BETWEEN  $\text{CaO-MgO-K}_2\text{O}$  FOR THE MAXIMUM (BUT NOT OPTIMUM) TREATMENT.—In figures 2 to 4 are plotted in trilinear coordinates the *Ca-Mg-K-unit* previously defined for the fourth leaf of plants growing on the plots indicated.

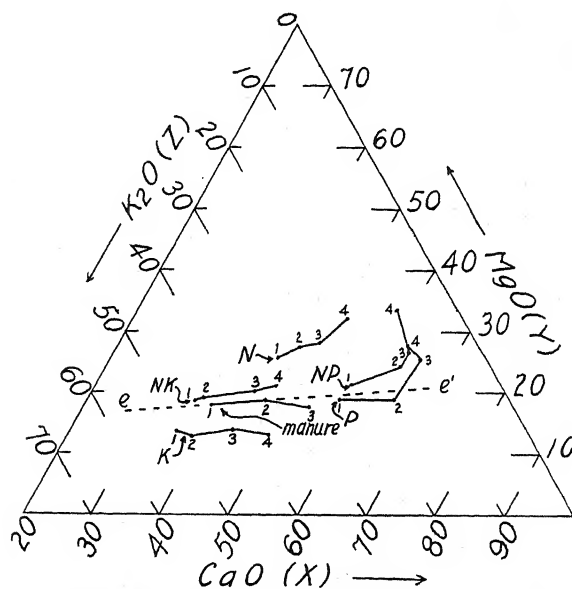


FIG. 2. Deviations from the optimum physiological balance (the line  $ee'$ ) between  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{K}_2\text{O}$  resulting from the treatments indicated (potato experiments).

The broken line  $ee'$  is drawn through the coordinate values for July 2 and July 16 of the highest yielding plot—*viz.*, manure (no. 1-15). The line joining the coordinate points for July 2 and July 16 has been extended and taken as the evolutionary course of the  $\text{CaO-MgO-K}_2\text{O}$  equilibrium of the fourth leaf during the growth cycle. The assumption is made that this would represent the course of nutrition as represented by the *Ca-Mg-K-units* if the plants had not been arrested in development at about August 20th as the result of unexpected contingencies, since we have shown that under optimum conditions, the relationship among  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$  is definitely linear in the case of *Zea mays*. The general conclusions made below will not be affected by this assumption.

Using the same coordinate nomenclature as before—viz.,  $CaO = (X)$ ,  $MgO = (Y)$ , and  $K_2O = (Z)$ , the equation of this line for manure (no. 1–15) is  $Y = 0.115X + 13.69$ .

CHARACTERISTICS OF THE GRAPHS OF THE *Ca-Mg-K-units* FOR THE VARIOUS TREATMENTS.—The treatments may be grouped as follows according to their position with respect to the line  $ee'$ ; that is, comparison is made with the graph of the maximum yielding plot (manure, plot no. 15).

*Group 1.*—NK (no. 1–7), K (no. 1–4) (figure 2): are distinguished from all other treatments in that these are the only two treatments that result in relative excess of  $K_2O$  in the *Ca-Mg-K-unit*. The displacement is slightly to the left (below  $ee'$  in [K] and above in [NK], indicating a small deficiency (relative) of  $CaO$  and a slight excess of  $K_2O$ . A deficiency of  $MgO$  exists in [K] and an excess in [NK].

*Group 2.*—N (no. 1–2), P (no. 1–3), NP (no. 1–5) (figure 2): Displacement is to the right, indicating excess (relative) of  $CaO$  and deficiency of  $K_2O$ . In [P] and [NP], balance with respect to  $MgO$  nearly exists in the young leaf, but the disequilibrium becomes greater and greater with advancing age.

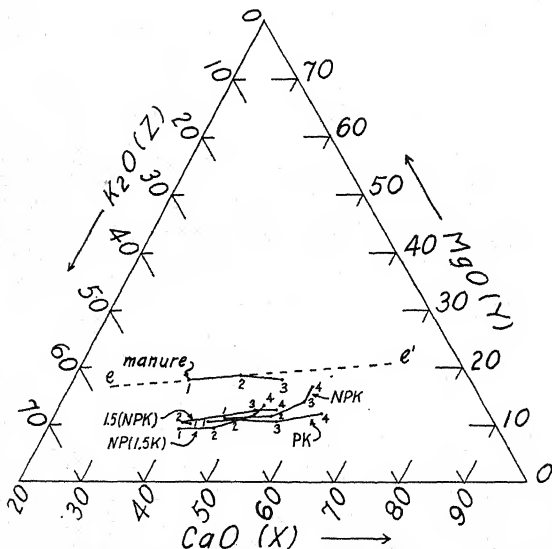


FIG. 3. Deviations from the optimum physiological balance (the line  $ee'$ ) between  $CaO$ ,  $MgO$ , and  $K_2O$  resulting from the treatments shown (potato experiments).

*Group 3.*—NPK (no. 1–10), PK (no. 1–7), (figure 3): Displacement is towards the right base apex and below the line  $ee'$ , indicating in the *Ca-Mg-K-unit* deficiency of  $MgO$  and slight excess of  $CaO$ .

*Group 4.*—NP (1.5K) (no. 2–12), 1.5(NPK) (no. 2–15) (figure 3): Displacement is towards the right base apex and below the line  $ee'$ , indicating in

the *Ca-Mg-K-unit* deficiency of MgO and slight excess of CaO and slight deficiency of MgO and slight excess of CaO and slight deficiency of K<sub>2</sub>O.

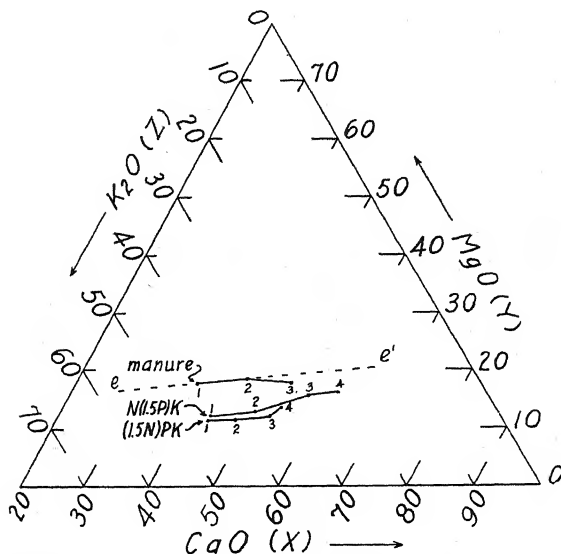


FIG. 4. Deviations from the optimum physiological balance (the line  $ee'$ ) between CaO, MgO, K<sub>2</sub>O resulting from the [N(1.5P)K] and [(1.5N)PK] treatments (potato experiments).

*Group 5.*—N(1.5P)K (no. 2-8); (1.5N)PK (no. 2-4) (figure 4). Displacement is to the right and below  $ee'$ , indicating in the *Ca-Mg-K-unit* deficiency of MgO, with slight excess of CaO.

DEPRESSING EFFECT OF CALCIUM ON THE ABSORPTION OF POTASH.—The positions of the graph of [P] and [NP] in figures 1 and 2, *i.e.*, of treatments containing superphosphate without potash indicates the poverty in K<sub>2</sub>O of the *Ca-Mg-K-unit* of the chosen leaf resulting from these treatments, showing that the effect of calcium in superphosphate cannot be ignored in field studies (12).

BEARING OF THESE RESULTS ON STUDIES IN HUMAN AND ANIMAL NUTRITION.—The results of this study suggest also that attempts to classify foods into acid-forming and base-forming from the analysis of the ash requires a knowledge of the conditions under which the plants were grown before any generalization with respect to a particular plant is possible.

### Summary

In this mysterious universe the planetary system of which is governed by laws that can be given mathematical expression, it is logical to hypothesize that the physiological processes of living organisms follow laws that

await discovery. In the normally functioning plant the relationship between nitrogen and phosphoric acid has been shown to be a linear function in time (6). The results of the present investigation indicate the law governing the absorption of the principal bases, lime, magnesia, and potash is, under optimum conditions, a linear function also. The experiments were at first conducted with maize growing on the Jordan fertility plots and subsequently with the potato growing on the vegetable fertility plots of the Pennsylvania Agricultural Experiment Station. In the former the third leaf and in the latter the fourth leaf from the base was sampled periodically during the growth cycle, following the method described earlier (7).

The equilibrium between  $\text{CaO-MgO-K}_2\text{O}$  on any sampling date is expressed as a composite *Ca-Mg-K unit* derived after the manner of the *NPK-unit* (7)—viz., the percentage composition for  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$  respectively at any given date is converted into milligram equivalent values and the proportion which each of these bears to the milligram equivalent total determined. The values so obtained are multiplied by 100 to avoid fractional values. The *Ca-Mg-K-units* so obtained are plotted in trilinear coordinates.

In the case of maize the line joining the coordinate points for July 6 to August 8 for the maximum yielding plot (manure+lime) is sensibly rectilinear in time. The deviations of the experimental values and the values calculated from the linear equation are given.

The significance of the equations relating the absorption of the three variables  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$  is discussed and their bearing on the work of earlier investigators is described.

The characteristics of the graphs for other plot treatments unlimed since 1881, together with a plot receiving lime only are discussed in relation to their deviation from the optimum graph (manure+lime).

The plots of the vegetable fertility experiments, in contrast, had received applications of ground limestone in amounts calculated to bring all the plots to approximately the same pH values. As a result the deviation from a linear function between the three bases is less marked for most treatments in the potato experiments. In the former the following treatments did not cause great deviation from the linear relationship:  $[\text{K}]$ ,  $[\text{NK}]$ ,  $[\text{PK}]$ ,  $[\text{1.5N(PK)}]$ ,  $[\text{N(1.5P)K}]$ ,  $[\text{NP(1.5K)}]$ , and  $[\text{1.5(NPK)}]$ ; nevertheless their position (as distinguished from form) relative to the graph of the highest yielding plot indicates that disequilibrium exists between  $\text{CaO-MgO-K}_2\text{O}$  in the plots. The characteristics of the graphs in relation to the graph from the highest yielding treatment, manure, are described.

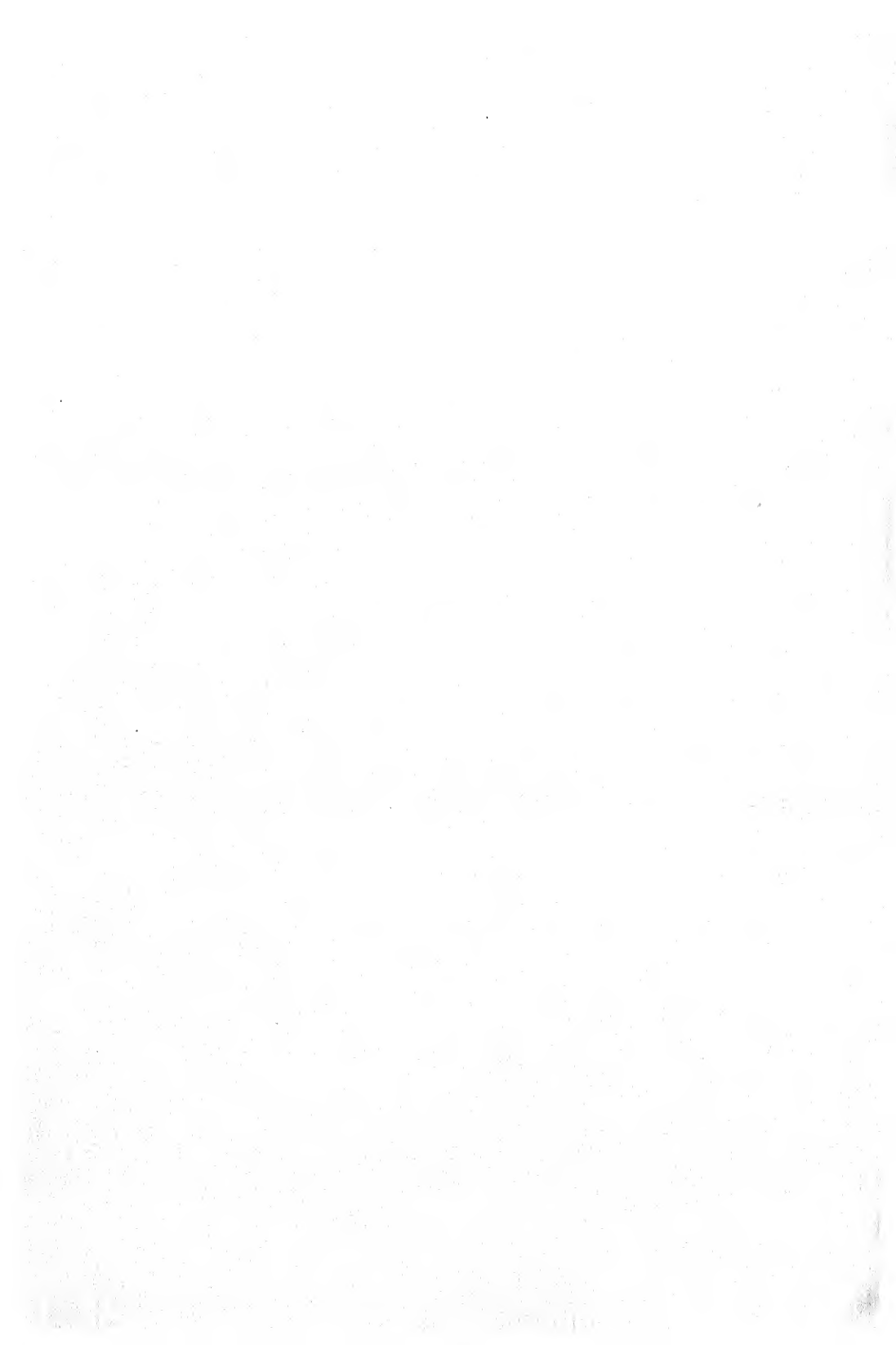
The evolutionary sequence of the composite *Ca-Mg-K-unit* constitutes a sensitive and faithful diagnosis of the mode of adaptation of the medium (soil) to the needs of the plant with respect to  $\text{CaO}$ ,  $\text{MgO}$ , and  $\text{K}_2\text{O}$ , when the respective graphs are interpreted in relation to the optimum treatment.

The bearing of the results on studies in human and animal nutrition is indicated.

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# EFFECTS OF CERTAIN INSECTICIDES AND INERT MATERIALS UPON THE TRANSPIRATION RATE OF BEAN PLANTS<sup>1</sup>

E. C. WAGNER

(WITH SEVEN FIGURES)

## Introduction

The changes in physiological processes of plants brought about by the application of spray materials are not well understood, nor have they been investigated in detail. That such changes do occur is evidenced by both the beneficial and the harmful effects of these compounds quite apart from their value as fungicides or insecticides. The increased growth and greater yield of potatoes sprayed with Bordeaux, the shot-hole effect of copper fungicides on peach leaves, the russetting of fruit and burning of foliage by arsenicals all point to profound changes in the physiology of the sprayed tissues.

In an attempt to determine the basis for the relative "safeness" of certain arsenates as compared with others, investigations into the effects of both kinds of sprays on the physiological processes in plants have been made. The process most widely studied in this connection has been transpiration. Some writers have suggested that the phytocidal action of certain sprays might be attributed, at least in part, to the sudden increase in the rate of water loss from the sprayed plants. Thus it was thought that perhaps a so-called "safe" compound which did not cause the typical burning of sprayed leaves, was innocuous because it did not bring about the increase in water loss which resulted when the injurious sprays were applied to the leaves.

Not all investigators are agreed as to the effect of sprays on transpiration. Many have recorded results in which there was no increase, but a decrease, or no change at all in the rate of water loss. In fact, it has been suggested that the stimulatory effect of Bordeaux on potatoes may be attributed to a conservation of the water supply.

The earlier workers investigated the effect of Bordeaux on crop plants. In general, their results showed a decrease in the transpiration rate of the sprayed plants as compared with that of the controls. Many of the data, however, were not very quantitative.

Some time later, DUGGAR and COOLEY (3), in studies of cut plants in potometers, found that Bordeaux increased the transpiration rate of castor bean, squash, *Phytolacca*, potato, and *Iresine*. They also studied the rates

<sup>1</sup> Papers from the Department of Botany no. 406, the Ohio State University. This work was done in cooperation with the Sherwin-Williams Co., and the Ohio State University Research Foundation.



of water loss from potted plants of potato and tomato with the same results. The tomato plants did not show an increase in transpiration resulting from the application of calcium hydroxide, clay, aluminum hydroxide, calcium carbonate, or lime-sulphur 1-25, but the potato plants lost more water when sprayed with lime water, Bordeaux and lampblack, lampblack alone, lime-sulphur, and lime wash, than did the control plants. Lime-sulphur did not cause an increase in water loss.

MARTIN (8) reported that data from potometer experiments with leaves of radish, bean, swiss chard, Hibiscus, Clerodendron, Caladium, Datura, and castor bean, showed that spraying with Bordeaux brought about a rapid increase in the transpiration rate, which later tended to return to the original rate. In some experiments with potted plants of tomato, cabbage, pepper, eggplant, and soybean, the rate of water loss was increased after the application of Bordeaux although this increase was more pronounced for the cut leaves in potometers. The effect was greatest during the first 2-hour period following application, and showed a variation in the amount of effect with the species.

SHIVE and MARTIN (10), using standardized cobalt chloride paper, studied the effect of Bordeaux on the daytime transpiration rate of tomato plants in the field and found that there was a decided increase after spraying. DUGGAR and BONNS (2), by weighing potted plants of potato, tomato, marguerite, and tobacco, obtained data which indicated that the increase in the rate of water loss resulting from the application of Bordeaux mixture occurred almost entirely during the night. Potted plants of *Cyperus esculentus* showed no increase. Leaves of castor bean in a potometer gave a continuously increased rate of transpiration, the increase not being confined to the night period. MARTIN and CLARK (9) experimented with potted potato plants grown in soils of three different moisture contents, and found that the greatest average increase in water loss occurred in the plants grown at the highest soil moisture content. WILSON and RUNNELS in several papers (12 to 19) report an increased transpiration rate of different species for plants sprayed with Bordeaux and other compounds. They also found that the greatest increase in water loss occurred at night. CHILDERS (1) found, on the contrary, no increase in the rate of water loss from potted tomato plants sprayed with Bordeaux. KRAUSCHE and GILBERT (7), in a study of tomato plants treated with copper sprays, reported an increase in the transpiration rate, especially at night. They could find no visible change in the stomata after spraying.<sup>2</sup>

Recently some experimentation has also been done on the effects of oil sprays on the transpiration of sprayed plants, from which data it is apparent

<sup>2</sup> See also HORSFALL, JAMES G., and HARRISON, A. L. Effect of Bordeaux mixture and its various elements on transpiration. Jour. Agr. Res. 58: 423-443. 1939, for additional data published since the completion of this paper.

that the application of oils brings about a reduction in the rate of water loss (5, 6). Very little work has been done on calcium arsenate in this connection.

### Methods

Two different methods of determining the rate of water loss before and after spraying were used. For both of these methods, the test plants were grown in the same way and used when at the same stage of development. The cranberry bean seeds were selected for uniformity in size, the average sized seeds being chosen; the large or small beans were discarded. They were soaked for six hours, drained, allowed to germinate in a moist atmosphere, and then planted in sand in paraffined trays watered with a cotton wick dipping into an attached water jar (11). All of the plants to be used indoors were kept in a culture room until used, while those to be used in the greenhouse were moved into it when the hypocotyls were about ten centimeters long. By indoors is meant a basement culture room kept at a constant temperature, 24° to 26° C., and a constant relative humidity of 50 per cent. Light was supplied by several 100-watt lights for 19 hours each day. The room was aerated with fans, but free from drafts, being divided into compartments which deflected the air currents. This room was an ideal place for experimentation with potometers. The plants were used for experimental purposes as soon as the first two leaves were fully expanded. The terminal bud was removed to prevent further growth of the plants, since they became very weak and spindly under the artificial light. In order to keep the experimental conditions uniform, this procedure was followed with the greenhouse plants as well.

### POTOMETER DETERMINATIONS

While doubts may be raised as to the quantitative value of data derived from studies of water absorption by cut plants in potometers, the fact remains that this method affords a means of determining rapid changes in the rate of water intake. With mature plants over short periods of time these changes in the rate of absorption should reflect rather accurately the rate of water loss from the leaves. That the transpiration rate of cut shoots differs quantitatively from that of intact plants cannot be denied, but experiments on different samples under the same environmental conditions should yield comparative data of value.

Four potometers were arranged under a light in a draft-free compartment in the constant temperature culture room. The bean stems bearing two well expanded unifoliate leaves were cut under water, inserted into holes in a rubber stopper which was quickly sealed into the potometer; two stems were used for each potometer. The different samples were allowed to stand for an hour. At the end of this period readings were made every

five (or in some cases, ten) minutes for an hour; the test materials were then applied and as soon as the leaves were dry, readings were again made for several hours. A duplicate series was run the next day, using fresh plants and changing the order of the potometers to obviate any effects of variations in the tubes or differences in the light conditions. Some tests with an atmometer, substituted for the bean shoots, showed that fluctuations caused by slight changes in the environmental factors were negligible. The amount of water loss from the atmometer was quite constant, showing none of the fluctuations characteristic of the curves which show the water absorption by bean stems.

Preliminary experimentation showed that variation in the method of applying the spray materials made no important differences in the order of the results obtained. A standard procedure was adopted which consisted of wetting the under surfaces of all of the leaves with distilled water from an atomizer, dusting the wetted areas with the materials to be tested, and then rewetting the dusted surfaces until droplets of a suspension formed. This method of application brought about the least injury with unsafe materials and eliminated any possible differences due to light effects on the whitened surfaces. In all of the experiments, one potometer contained check plants sprayed with water only, one contained plants treated with ordinary commercial calcium arsenate, and the two remaining were used to test various other materials. The materials tested were: zinc-safened, basic, and ordinary commercial calcium arsenates, lead arsenate, a solution of dicalcium arsenate, Bordeaux, copper sulphate, lime, and Bancroft clay. The ordinary commercial calcium arsenate gave a test for 5 per cent. water soluble arsenic when 2 grams in a liter of water were in equilibrium with the carbon dioxide of the air, and caused 100 per cent. injury on bean leaves kept wet for 2 hours after spraying. The basic calcium arsenate under the same conditions yielded 2.5 per cent. water soluble arsenic and brought about only moderate injury on sprayed bean leaves kept wet for two days, while the zinc-safened material showed 0.7 per cent. water soluble arsenic, and caused only slight injury after the sprayed leaves had been kept wet for two days after spraying.

Two checks on the changes in the rate of water absorption were possible. One was the rate of absorption of all of the plants before treatment with the test materials, the other was the rate of absorption of the check plants throughout the experiment. The importance of carrying out potometer experiments under as constant conditions as possible is quite apparent. For determinations over short periods of time, such as 5- or 10-minute intervals, air currents can cause a considerable error, which may not become apparent if the check samples are not touched by the moving air. Hence for such measurements, the environment outdoors, or even in the green-

house, was too variable from moment to moment to allow accurate readings to be made.

#### EXPERIMENTS WITH POTTED PLANTS

In order to obtain data showing the effects of spray materials over a longer period of time, some experiments were set up using bean plants grown in sand in paraffined trays fitted with bottles which furnished a constant water supply to the sand through a cotton wick (11). The water in the bottles was replenished as often as the level became low. At times there was a change of five centimeters in the water level. The roots appeared

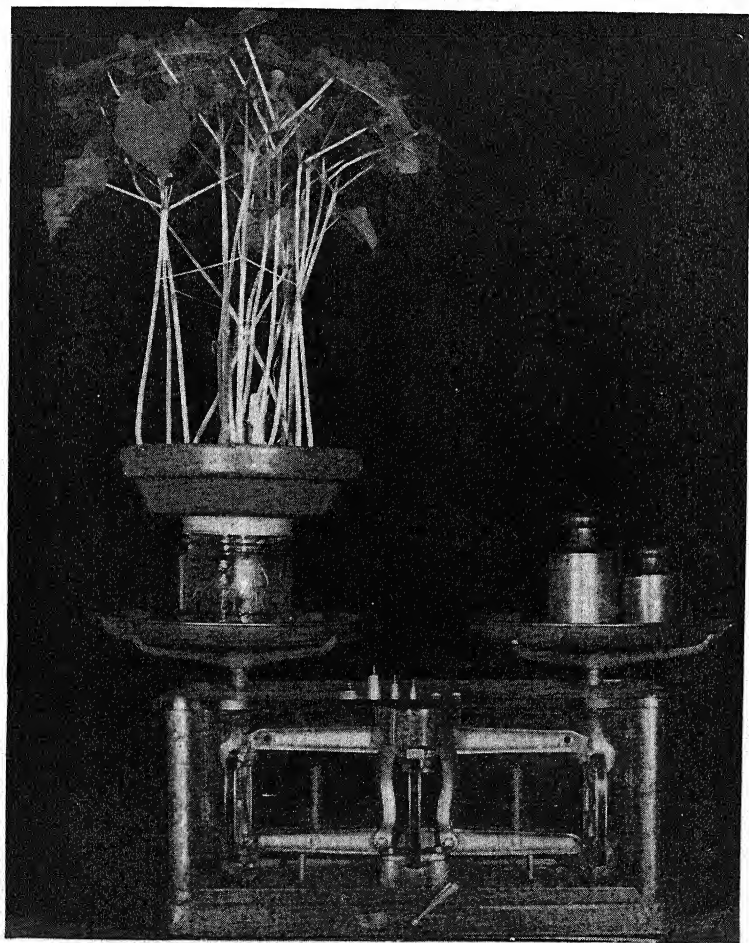


FIG. 1. Photograph of one of the trays of bean plants used in the weighing experiments to determine the amount of water lost by the plants.

healthy and apparently were not injured by the rather poor aeration of the sand. In the indoor culture room, the plants remained in good condition for from 2 to 3 weeks when the terminal bud was removed regularly. Figure 1 shows the tray with the attached water supply as the samples were weighed to determine the amount of water loss.

When the plants had fully matured, unifoliate leaves, the stand was thinned to 14 plants per tray, the sand covered with a paraffin-vaseline sealing compound, and the various trays arranged indoors or in the greenhouse. A control, set up in the same way but without plants, lost no weight over a period of several days. Two or three trays were used for each material tested, and, in addition, the experiment was repeated at least twice, each time with slight variations in procedure but showing no significant differences in the order of the results. The trays, including the jars of water, were weighed twice daily just after dark at night, and just before daylight in the morning. In the winter months, this was about 6:00 A.M. and 6:00 P.M. These data gave an indication of the effects of periods of light and darkness upon the transpiration rate of the greenhouse plants. A duplicate series was also run indoors and weighed at the same time; the plants had a longer light period indoors since the lights were on 19 hours a day.

The plants were weighed morning and evening for three or four days, before treatment with the spray materials, in order to get the relative rates of the various samples. The different trays were selected so that the samples used for each compound were assorted with one tray of plants showing a high transpiration rate, one an average rate, and one a low rate. An attempt was made to distribute the different samples at random, and to arrange them so that one of each set was surrounded by plants and the other two were at opposite edges of the table. A comparison of the rates of water loss from samples at different positions on the table did not reveal any correlation between the amount of transpiration and the position of the tray. As a precautionary measure, however, the random distribution was used.

After treatment with the test materials, the plants were weighed for ten days or two weeks afterwards depending upon the condition of the plants. There was some increase in leaf areas of the unifoliate leaves under greenhouse conditions but it appeared to be approximately equal for all of the samples. In one series, the terminal bud was allowed to grow, with the result that the plants differed considerably in amount of growth; the ones sprayed with zinc-safened calcium arsenate showed the greatest growth and made such a difference in leaf areas that the transpiration rates of the different samples could not be compared. A certain amount of injury with the ordinary commercial calcium arsenate was unavoidable, but was kept at 5 per cent. or less. The materials were applied as dusts to the wetted under-

surfaces of the leaves as in the potometer experiments. The compounds tested were: zinc-safened, basic, and ordinary commercial calcium arsenates, a solution of dicalcium arsenate, copper sulphate, lime, silica,<sup>3</sup> and Bancroft clay.

Some tests using cobalt chloride paper on bean plants in the field were set up to determine the time when the night changes in water loss occurred. The data, however, were difficult to interpret and yielded no significant

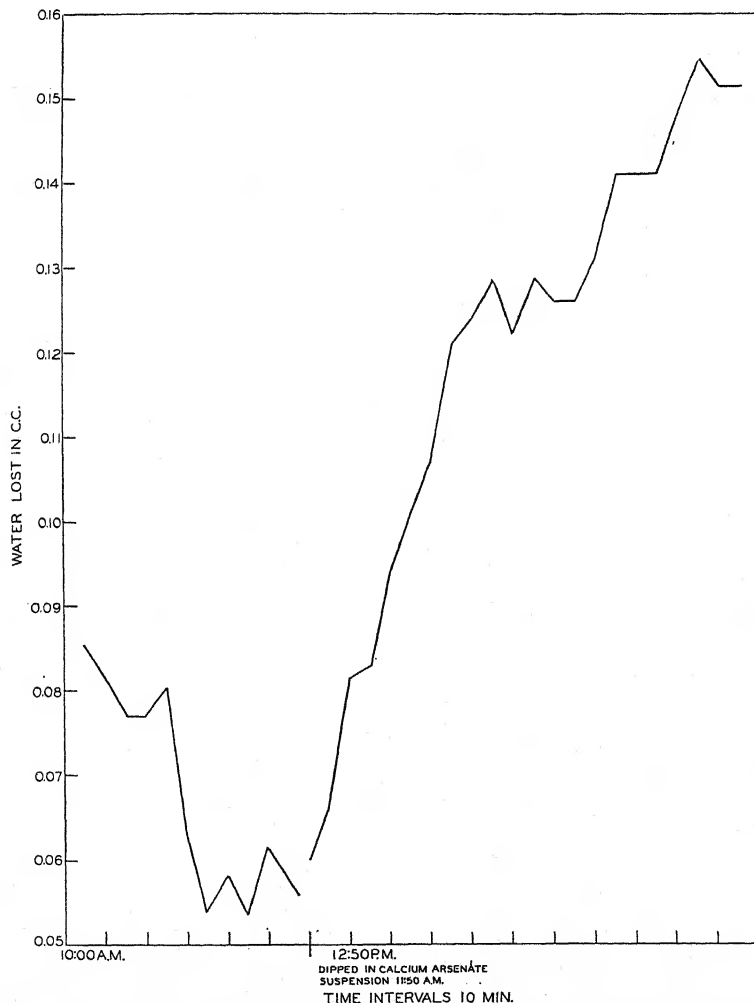


FIG. 2. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial calcium arsenate.

<sup>3</sup> The words silica and silicon dioxide are used interchangeably, both referring to the pure compound silicon dioxide in powdered form.

information. Several errors were possible. One was the effect on the cobalt chloride paper of the material dusted on the leaves, and another was the error introduced by the presence of condensed moisture on the leaves at night. In addition, the color change was difficult to determine with artificial light. Since the data from the other methods showed such uniformity of results, it was felt that this method was in error rather than that the other data were invalidated.

### Results

Figure 2 shows the immediate increase in the rate of water absorption of the bean shoots after treatment with ordinary commercial calcium arsenate. This result was characteristic of all the materials tested. Whether the effect was to increase water absorption or to decrease it (as in the case of copper sulphate), the change in rate was apparent as soon as the leaves had dried. The results shown in figure 3 indicate that the zinc-safened

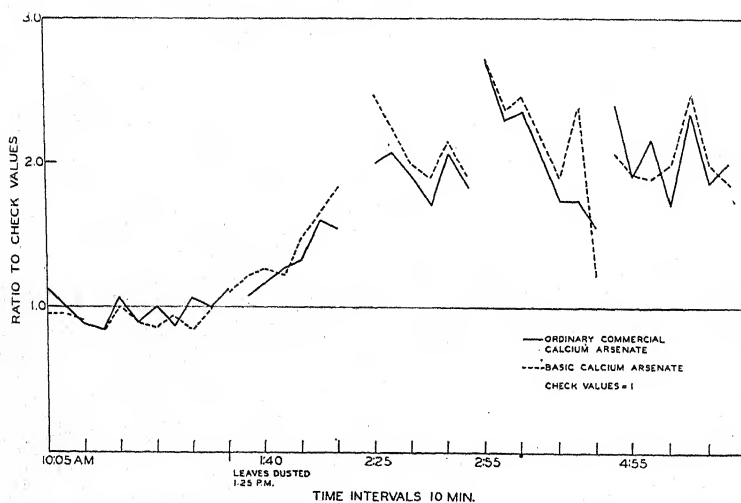


Fig. 3. Potometer curve of the rate of water absorption by bean shoots before and after treatment with ordinary commercial and basic calcium arsenates.

calcium arsenate brought about nearly the same amount of increase in water absorption as did the ordinary commercial material when both are compared with controls sprayed with water. For the preceding graph and for those that follow, the check values were reduced to unity in order to reduce the number of graphs per figure and to show the results more clearly. For these calculations, the average number of grams of water lost by the check samples was divided into the average number of grams of water lost by the samples treated with any one material for that same period of time. In this way a correction was made for weather changes such as

rain following sunshine, as well as for the physiological changes taking place in the plants with the passage of time. The check value, therefore, became a straight line. The deviations from the line by the other curves should indicate the relative amount of change brought about by the application of the test materials.

Other materials which brought about increases in the rate of water absorption as measured by potometers were lead arsenate, Bordeaux, hydrated lime, and Bancroft clay. The application of copper sulphate under the same conditions resulted in a decrease in the rate. During the course of

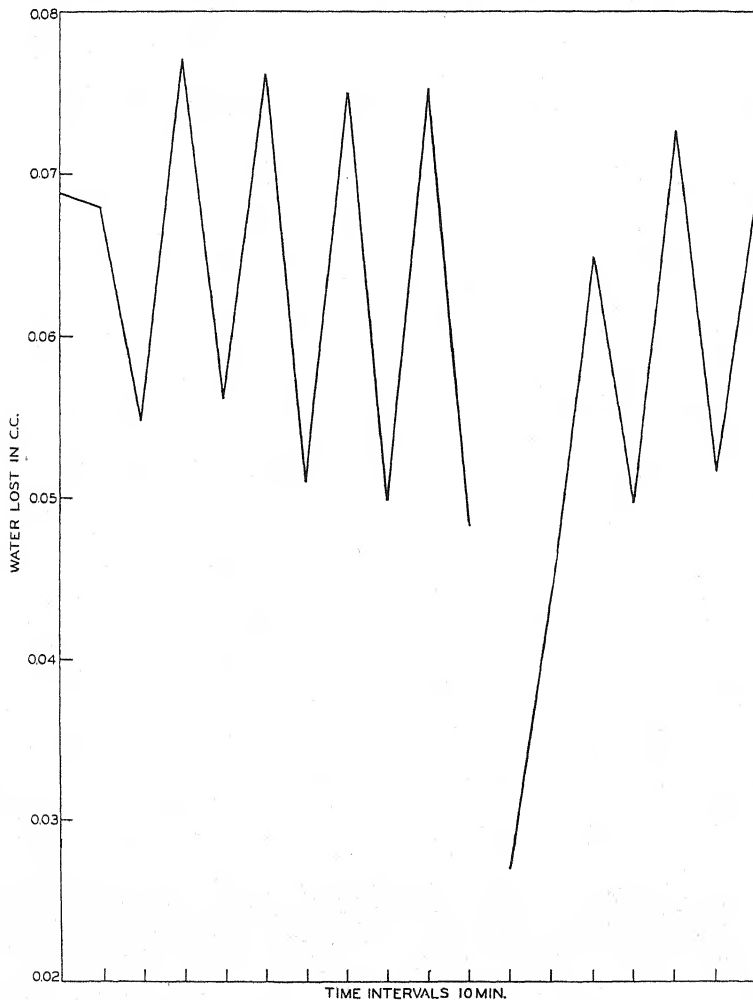


FIG. 4. Potometer curve showing rhythm in the rate of water absorption by two shoots of the check plants.



experimentation with cut plants in potometers, some curves of water absorption were obtained which showed unusual rhythmic fluctuations (fig. 4). Tests with an atmometer in place of the bean stems showed that these variations were not a result of changing environmental conditions, such as air currents, nor of physical forces involved in the movement of a bubble down the tube, for the atmometer curves showed no such variations. Apparently

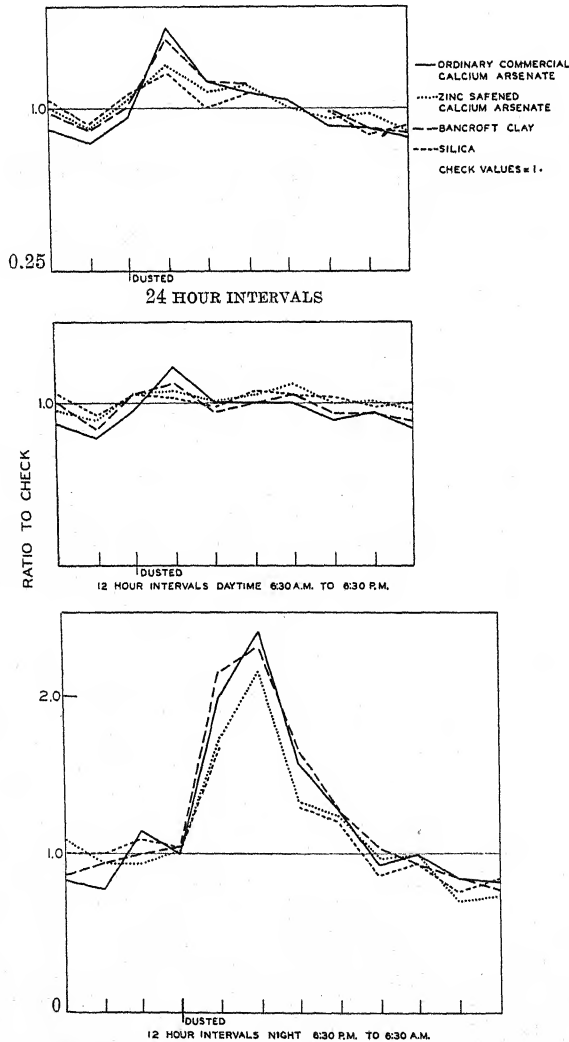


FIG. 5. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the greenhouse.

the fluctuations were brought about by physiological changes within the plants,—perhaps a lag of water absorption by the stem behind the water loss from the leaves. Sometimes the data from two samples run on the same day would show these variations, with one curve showing maximum where the other showed minimum.

Figure 5 and table I show the comparative effects of the zinc-safened and ordinary calcium arsenates, Bancroft clay, and silica, on the amount of water loss from potted bean plants in the greenhouse. The table is included to show the absolute amount of water lost by the different samples. The amounts of loss for the 24-hr. period and the 12-hr. periods of daylight and darkness show that the greater change occurs during the night period. These results are in accord with those of DUGGAR and COOLEY (4), and WILSON and RUNNELS (12). The total amount of water lost by the treated plants for the 24-hr. period was not much greater than that lost from the check plants (for the greenhouse samples), which fact may account for some of the discrepancies in the literature. The 24-hr. measurements might not reveal the great increase in transpiration of the sprayed plants at night as compared with the checks, since the total number of grams of water lost by any of the samples at night is usually so much less than the amount lost during the daytime that the changes might not be apparent.

The curves also show that such materials as silica and Bancroft clay can bring about increases as great as those resulting from the application of ordinary spray materials. To ascertain whether or not these results might be attributed to some chemical contamination of the silica and Bancroft clay, another series was run, using these materials washed until chemical tests showed scarcely a trace of any other materials present. The results were the same for the second series. After several days the rates of all of the treated plants began to approach the original rates of water loss.

The curves (fig. 6) and data in table II show the effects of the same materials tested on a similar series of plants kept indoors in the culture room. The high rate at one time before treatment is apparently caused by an error in recording the loss in weight of the check plants. Here there is no pronounced difference between the daytime and the night losses. These plants had only 5 hours of darkness, being illuminated for 19 hours every day with electric lights. These curves show a separation into pairs, the ordinary commercial calcium arsenate and the Bancroft clay bringing about approximately the same increases, and the zinc-safened calcium arsenate and the silica showing somewhat similar effects. Here the application of a zinc-safened material did not bring about as great an increase in the transpiration rate as did the ordinary commercial calcium arsenate. The effect of the latter, however, may be somewhat exaggerated, because the plants in these samples showed as high as 10 or 20 per cent. injury. What the water

TABLE I  
LOSS IN WEIGHT OF POTTED BEAN PLANTS IN THE GREENHOUSE BEFORE AND AFTER TREATMENT WITH CERTAIN MATERIALS\*

WATER LOSS IN 24 HOURS (AVERAGE OF TWO TRAYS)													
SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN. 11†	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19 20
Ordinary commercial calcium arsenate .....	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Zinc-safened calcium arsenate .....	.....	65	80	58	.....	99	91	92	98	84	105	93	79 104
Bancroft clay .....	.....	66	83	58	.....	79	82	84	95	81	102	87	72 83
Silicon dioxide .....	.....	76	90	63	.....	86	87	94	104	88	102	93	83 101
Check .....	.....	78	92	65	.....	82	86	89	97	90	101	95	83 80
	.....	72	101	61	.....	66	79	83	91	88	110	102	86 90
WATER LOSS DURING THE PERIOD 6: 45 A.M. TO 6: 45 P.M.													
Ordinary commercial calcium arsenate .....	.....	54	70	48	.....	55	74	69	79	63	65	67	53 61
Zinc-safened calcium arsenate .....	.....	56	72	48	.....	46	68	65	78	62	65	67	50 58
Bancroft clay .....	.....	62	78	53	.....	43	71	70	81	66	65	70	59 58
Silicon dioxide .....	.....	62	81	54	.....	48	73	72	80	71	65	72	58 61
Check .....	.....	61	88	50	.....	46	72	68	76	66	66	73	56 57
WATER LOSS DURING THE PERIOD 6: 45 P.M. TO 6: 45 A.M.													
Ordinary commercial calcium arsenate .....	11	10	10	10	44	17	23	19	21	41	26	26	43 46
Zinc-safened calcium arsenate .....	10	11	10	10	33	14	19	17	19	37	20	22	25 29
Bancroft clay .....	14	12	10	11	43	16	24	23	22	37	23	24	43 41
Silicon dioxide .....	16	11	11	15	34	13	19	17	19	36	23	25	19 53
Check .....	11	13	11	10	20	7	15	15	22	44	29	30	33 50

\* Blank spaces indicate that the weighings were omitted at the beginning of the experiment and during the day on which the plants were treated with the test materials.

† The plants were treated with the test materials during the afternoon.

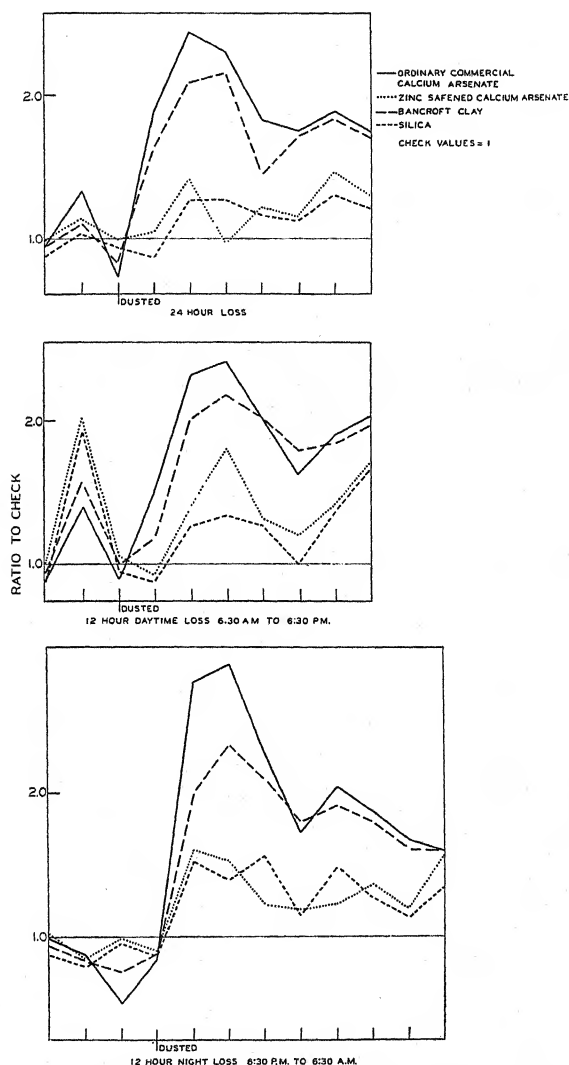


FIG. 6. Relative amounts of water lost from check plants and plants treated with certain materials applied as dusts to the wetted leaves. The test samples were potted bean plants in the indoor culture room.

loss from such injured areas may be has not been determined. For these plants the increased rates of water loss do not tend to return to the original rates.

A comparison was then made of the effects of washed Bancroft clay and silica suspended in water and in a solution of dicalcium arsenate containing one-half per cent. water-soluble arsenic. The leaves were dipped

TABLE II  
LOSS IN WEIGHT OF POTTED BEAN PLANTS IN THE INDOOR CULTURE ROOM BEFORE AND AFTER TREATMENT WITH CERTAIN MATERIALS\*

WATER LOSS IN 24 HOURS (AVERAGE OF TWO TRAYS)														
SAMPLE	JAN. 7	JAN. 8	JAN. 9	JAN. 10	JAN. 11	JAN. 12	JAN. 13	JAN. 14	JAN. 15	JAN. 16	JAN. 17	JAN. 18	JAN. 19	JAN. 20
Ordinary commercial calcium arsenate .....	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Zinc-safened calcium arsenate .....	.....	21	22	13	.....	47	44	39	34	31	30	29	28	35
Bancroft clay .....	.....	24	19	17	.....	29	26	20	22	19	21	25	23	18
Silicon dioxide .....	.....	28	27	14	.....	46	38	37	35	31	29	31	34	36
Check .....	.....	26	17	20	.....	28	23	21	21	20	21	17	24	24
Check .....	.....	22	21	17	.....	25	18	17	18	18	17	19	24	21
WATER LOSS DURING THE PERIOD 6: 45 A.M. TO 6: 45 P.M.														
Ordinary commercial calcium arsenate .....	.....	12	13	8	.....	22	22	20	18	15	14	15	14	17
Zinc-safened calcium arsenate .....	.....	12	10	8	.....	14	13	10	11	10	10	12	10	10
Bancroft clay .....	.....	12	15	7	.....	28	19	19	18	16	14	16	18	22
Silicon dioxide .....	.....	11	9	12	.....	14	12	11	11	9	10	7	12	13
Check .....	.....	12	11	8	.....	16	10	9	9	10	8	9	14	11
WATER LOSS DURING THE PERIOD 6: 45 P.M. TO 6: 45 A.M.														
Ordinary commercial calcium arsenate .....	9	9	5	7	25	22	19	16	16	16	14	14	18	14
Zinc-safened calcium arsenate .....	12	9	9	8	15	13	10	10	9	11	10	13	13	10
Bancroft clay .....	16	12	7	8	18	19	18	17	15	15	15	16	14	18
Silicon dioxide .....	15	8	8	11	14	11	10	10	11	11	10	12	11	12
Check .....	10	10	9	8	9	8	8	9	8	9	10	10	10	11

\* The blank spaces indicate that weighings were omitted at the beginning of the experiment and during the day on which the plants were treated with the test materials.

† Treated with test materials during the afternoon.

in a 2-100 suspension. The silica was further separated into coarse and fine particles by allowing it to settle in water. An attempt was made to separate out the particles smaller than the stomatal aperture of the bean leaf from those larger. It is apparent from the curves shown in figure 7

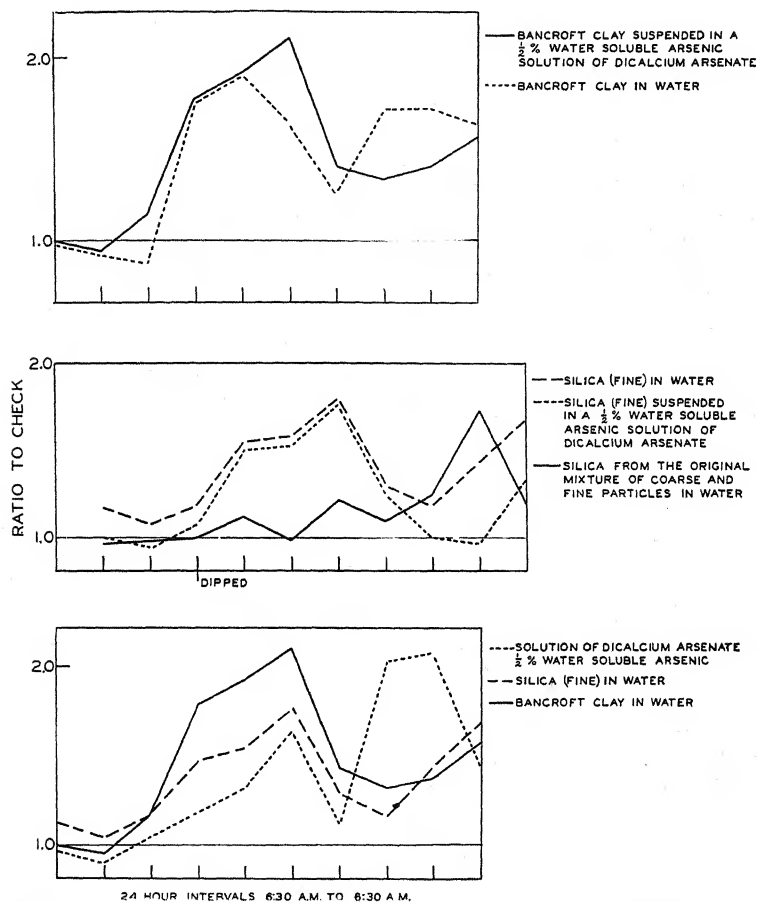


FIG. 7. Relative amounts of water lost from check plants and plants dipped in suspensions of the test materials. The samples were potted bean plants in the indoor culture room. Check value = 1.

that particle size affects the amount of change in the rate of water loss. It is also obvious that chemically inert materials can bring about quite as large increases in the transpiration rate as physiologically active materials such as dicalcium arsenate.

### Discussion

From the above results, several interesting conclusions may be drawn. In the first place, it is apparent that the relative safety of one calcium

arsenate over another is not correlated with its effect on water loss. Second, chemically inert and innocuous materials such as Bancroft clay and silica, as well as such a physically inert material as soluble dicalcium arsenate, can both bring about very significant increases in the transpiration rate. Particle size is a factor affecting the amount of increase. Third, for greenhouse plants, the greatest relative increase in water loss of the treated plants occurs at night.

The question then arises as to the nature of the action of these materials which bring about such changes in the transpiration rate. Very few explanations have been offered in the literature. Early workers suggested the effects of the white coating on light absorption, leaf temperatures, or photosynthesis. These factors could not have entered into the problem here because the test materials, with one exception, were always applied only to the undersurfaces of the leaves. DUGGAR and BONNS (2) suggested the influence of surface films on water loss from the stomata and hydathodes since they also found a decrease in the effect with time. KRAUSCHE and GILBERT (7) thought there must be an effect on the cuticular transpiration.

It is difficult to see what changes in cuticular transpiration could take place after the application of chemically inert materials in the form of dusts. It is hardly possible that compounds like chemically pure silicon dioxide could change the permeability of the epidermal cells to such an extent that more water could pass through the walls, especially when they are applied in such a manner as to prevent injury to the leaf. Since differences in particle size brought about significant differences in the amount of change in the rate of water loss, it appears that perhaps an effect on the stomata might explain the facts more easily.

It is significant that the greatest difference in the amount of water loss between the treated plants and the check plants in the greenhouse should occur at night. Since cuticular transpiration presumably continues at all times during the 24-hr. period, and is not influenced by the presence or absence of light as is stomatal transpiration, any differences in this rate should be apparent in the daytime as well as the night. It should also show up as a greater increase for the 24-hr. period as well. From these data, this is not the case. The daytime, and even at times the 24-hr. increase in rate, is almost negligible for the greenhouse plants. Even though the amounts of water lost at night are small, it seems that there should be more of a difference than there is in the daytime rate since the increases would be cumulative for the entire 24 hours. On the other hand, if one postulates that the effect is on stomatal transpiration, it is easy to account for the fact that the increase is always greater at night and almost negligible during the day, when the stomata would be open anyway.

Examination of the dusted leaves with a microscope showed particles

partially blocking the stomata. This observation, coupled with the fact that the smaller sized particles brought about a greater increase in the transpiration rate, indicates that the blocking of stomatal openings by these particles might prevent complete closure of the stomata. Since not all of the stomata would be blocked, necessarily, the rate of water loss would not be expected to approach the daytime rate; it would, however, be greater than the cuticular loss alone. That the greenhouse plants outgrow, so to speak, the effect of these materials, seems to be a result of an increase in area of the sprayed leaves, maturation of undeveloped stomata, and a gradual loss of the spray particles. Thus transpiring areas unaffected by previous treatment are acquired by the plant.

When indoor plants were used in the same experiment, there was neither the great change in the night rate of transpiration, nor the gradual return of the increased rates to the original values. The first effect appears to be related to the day-night rhythm of transpiration effective in the greenhouse, and the second to the increase in area of the sprayed leaves which does not take place indoors under artificial light. Under these conditions, the accelerating effect of the inert materials (Bancroft clay and silica) upon the rate of water loss is still more difficult to explain, even when taken into consideration as effect on the stomata. Since there are no data available on the condition of the stomata in plants grown under these conditions, and one does not know whether or not the artificial light can affect the opening and closing of the stomatal apertures, it is impossible to speculate as to what the effect may be until more data are obtained. As in the case of the greenhouse plants, it is difficult to account for the effect of chemically inert materials on the amount of water passing through the cuticle.

That there is a chemical, as well as a mechanical effect, is evidenced by the fact that a solution of dicalcium arsenate will also bring about increases in the rate of water loss of the treated plants. There are two possible explanations of this result. Either there is a chemical effect on the cell walls, rendering them more permeable and thus increasing cuticular transpiration, or there is an effect on the guard cells of the stomata which causes the stomata to remain open longer. It is known that the guard cells differ from the other epidermal cells in physiological activity. The fact that certain workers have reported a greater increase in transpiration when the leaves were sprayed on the lower surfaces as compared with leaves sprayed on the upper surfaces, also indicates that the action of these chemically active materials may be on the stomatal responses rather than on cuticular permeability, since the plants studied had more stomata on the lower epidermis than on the upper. An investigation into the condition of the stomata of the sprayed and unsprayed plants at all times of the night and day will be necessary before an explanation of these effects of sprays and other materials can be based on direct evidence rather than inferred from experimental data.



### Summary

Zinc-safened, basic, and ordinary commercial calcium arsenates, chemically pure Bancroft clay and silica, and a solution of dicalcium arsenate all brought about significant increases in the transpiration rate of bean plants, as determined by both a study of cut shoots in potometers and the loss in weight of potted plants.

The application of zinc-safened and basic calcium arsenates brought about as great an increase in the rate of water loss of the treated plants as did the ordinary commercial material. In this case the phytocidal action of a compound is not correlated with its capacity to increase the transpiration rate of the injured plants. If injury becomes apparent, however, as burned areas on the leaves, these plants will show a higher rate of water loss than the uninjured plants.

For greenhouse plants, the increased rate of water loss was most apparent at night when the treated plants were compared with controls. This was not the case for the plants kept in the indoor culture room.

Chemically inert Bancroft clay and silica, as well as physically inert dicalcium arsenate in solution, brought about equally large increases in the transpiration rate of the treated plants as compared with controls.

An attempt is made to explain some of the results on the basis of changes in stomatal action brought about by the application of the different materials studied.

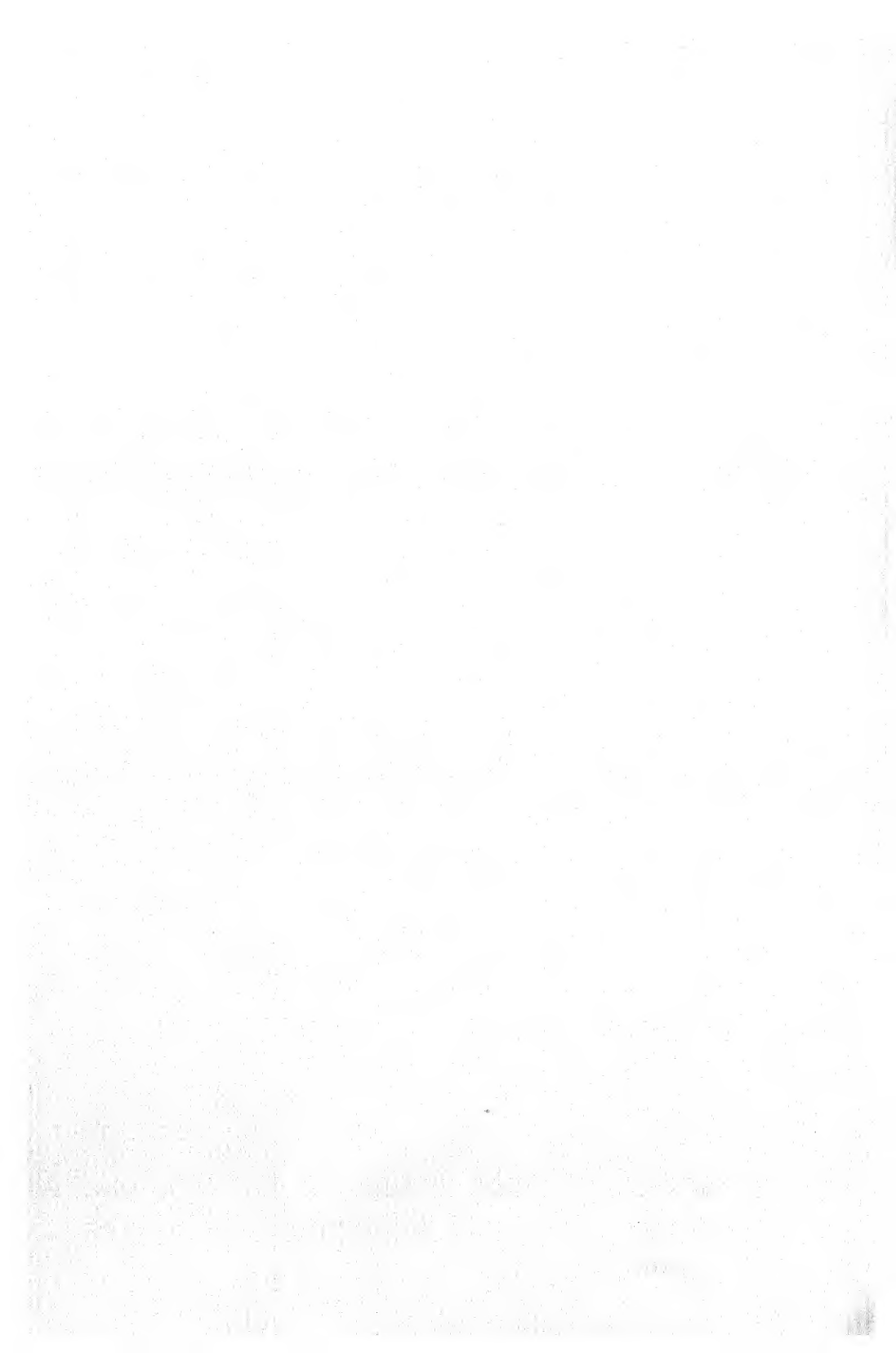
Grateful acknowledgment is made of the helpful suggestions and advice given by Prof. D. M. DeLONG of the Zoology and Entomology Department, and Dr. B. S. MEYER of the Botany Department, of the Ohio State University, and to Dr. H. A. WATERS of the Sherwin-Williams Research Project.

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# POLYSACCHARIDES OF THE VEGETATIVE TISSUES OF MAIZE<sup>1</sup>

C. G. BARR

(WITH ELEVEN FIGURES)

## Introduction

The polysaccharides of the vegetative tissues of maize have been designated as starch or starch-like by a number of investigators (14, 16) who have considered acid hydrolyzable materials to be starch. COLIN and DE CUGNAC (2), however, found that sugars, dextrins, and levulins are the characteristic reserves of the vegetative plants of the Gramineae, even of the cereals in which starch is the reserve polysaccharide of the fruit. LOOMIS (11) also found that polysaccharides do not constitute an important fraction of the vegetative maize plant, even under conditions favorable to maximum accumulation of reserves within the plant. The study of the polysaccharides of maize involves, then, the more exact determination of the compounds present, as well as their relation to photosynthesis, translocation, and growth within the plant.

## Materials and methods

The material used was obtained from a selection of open pollinated Reid's Yellow Dent maize. Samples were taken at 3-hr. intervals beginning at 4 A. M. and continuing for 24 hours, unless otherwise indicated. From 15 to 20 stalks were selected at random from a row of 60 hills at each sampling. The middle portion of each stalk bearing two leaves was brought quickly to the laboratory, where the leaves were removed and distributed into two groups so that an upper leaf from each alternate stalk was contained in each group. After the leaves were split down the midrib, duplicate 100-gm. samples of the blades were weighed to  $\pm 0.10$  gm. Each sample was then cut over and allowed to fall into a quart Mason fruit jar containing 450 ml. of boiling 95 per cent. alcohol. The quantities of alcohol were such as to give a final concentration of 80 per cent. when diluted by the water in the samples. After simmering for about 30 min. the jars were sealed and stored.

Duplicate samples of the stalk sections from which the leaves had been removed were taken also. The section of the stalk used for sampling was obtained from the first internode above the ear, or as near this point as possible in the first samplings, which were made before the ear shoot showed

<sup>1</sup> Journal paper no. J-643, Iowa Agricultural Experiment Station. Project no. 545. Preliminary work on these studies was aided by a grant from the Rockefeller Fluid Research Fund.

above the leaf sheath. The ends of the internode were cut away and the central portion of pith and rind quartered longitudinally. One-hundred-gram samples were cut into 2- to 4-mm. pieces and allowed to fall into jars containing 450 ml. of boiling 95 per cent. alcohol. These were boiled gently on a steam bath for 30 to 40 min., sealed, and stored.

Soluble carbohydrates were extracted with 80 per cent. alcohol before the polysaccharide analyses were begun. The residues from the 80 per cent. alcoholic extraction were dried to constant weight under reduced pressure at 65° C. The dried residues were ground in a Wiley and a ball mill until 98 per cent. or more of the sample would pass through a 200-mesh sieve.

The polysaccharides were extracted from this ground residue by various methods. Normally the first extraction was made with cold 10 per cent. alcohol (9), the second with hot water, the third with saliva, and the fourth with boiling 1+20<sup>2</sup> HCl. The first three extracts were cleared, made to volume, filtered, dealeded, aliquots hydrolyzed with HCl (1+20), and the reducing value of the extract determined. Clearing was omitted for the acid-hydrolyzable extract which was cooled, neutralized, made to volume and tested for reducing substances. Clearing was also omitted from some of the water extracts, or aliquots of these extracts, to determine the reducing value of the clearing precipitate.

Hydrolysis of the extracts was accomplished by refluxing, sometimes on an open flame turned low and sometimes in a boiling water bath, or by autoclaving with 1+20 or 1+40 HCl at 120° C. When the method of hydrolysis is not indicated, 1+20 HCl at 120° C. for one hour was used. The results are expressed in the main as percentage glucose equivalent on the basis of green weight. In the extraction and hydrolysis experiments, the results are expressed as milligrams of glucose equivalent per 50 ml. of uniform aliquots.

## Experimental results

### FRACTIONATION OF THE POLYSACCHARIDES OF MAIZE

In the absence of published methods for the fractionation of the polysaccharides of maize it was necessary to perform a number of experiments for the development of suitable methods. Eight 1.25-gm. samples of a composite lot of powdered, sugar-free maize leaf tissue were weighed carefully and transferred to 50-ml. centrifuge tubes. Thirty to 40 ml. of cold 10 per cent. alcohol was then added and allowed to stand 20 min. with occasional stirring. The samples were divided into lots of 4 each and carried through in a duplicate series of extractions. Two successive extractions from the 4 tubes were combined in 250-ml. volumetric flasks for each

<sup>2</sup> One part concentrated HCl and 20 parts extract by volume.

determination. Aliquots of the extracts were cleared and prepared for hydrolysis as described above. Fifty-ml. portions of the cleared and un-cleared solutions were then tested for reducing substances which were calculated as mg. of dextrose per 50-ml. aliquot. The results of the tests on the different extractions are shown in table I. A heavy yield of reducing substances was obtained in the combined first and second extractions. The third and fourth extractions dropped to a low yield, which continued with slight decrease and some fluctuation through the eighteenth extraction. The first four extractions were considered to have removed a distinct polysaccharide fraction which was designated as the alpha fraction.

The residue in the centrifuge tubes after the cold 10 per cent. alcohol extractions was covered with 30 to 40 ml. of boiling distilled water, the rack containing the tubes was placed in a boiling water bath for 20 min. and the contents stirred at intervals. Successive extractions were combined, aliquots cleared, and the cleared and un-cleared solutions tested for reducing substances. A second fraction yielding reducing substances on acid hydrolysis was removed by the hot water extraction. Normally this fraction, designated as the beta fraction, was smaller than the first although in drier seasons the beta fraction was of equal or major importance.

TABLE I

EXTRACTION OF ALPHA AND BETA POLYSACCHARIDE FRACTIONS FROM MAIZE LEAF POWDER

EXTRACTIONS	ALPHA FRACTION			BETA FRACTION		
	GLUCOSE VALUES*	GLUCOSE VALUES* TOTALS		GLUCOSE VALUES*	GLUCOSE VALUES* TOTALS	
	CLEARED	CLEARED	UNCLEARED	CLEARED	CLEARED	UNCLEARED
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
1 and 2 .....	27.30	27.30	33.50	6.40	6.40	9.60
3 and 4 .....	0.43	27.73	35.45	1.70	8.10	12.20
5 and 6 .....	0.40	28.13	36.70	0.70	8.80	13.35
7 and 8 .....	0.20	28.33	37.95	1.15	9.95	14.45
9 and 10 .....	0.00	28.33	38.15	0.20	10.15	14.80

\* Mg. glucose equivalent per 50 ml. of uniform aliquots.

The glucose equivalent values of the first 10 extractions for alpha and beta polysaccharides are shown in table I. As already indicated, continued extractions gave fluctuating low values suggesting slow hydrolysis of the substrate. The beta fraction was not only insoluble in cold 10 per cent. alcohol, but was rather slowly extracted by boiling water. The first 2 cold extractions removed 96 per cent. of the material obtained in 10 extractions, whereas the first two hot extractions removed only 63 per cent. of the total. Other differences in the two fractions are discussed later.

The beta polysaccharide fraction gave a brown color with I-KI solution,

even when concentrated by precipitating from solution with alcohol, and was in consequence considered to be starch-free. The residue from the 10 per cent. alcohol and hot water extractions was gelatinized, treated with tested saliva, and analyzed for starch by the method given by LOOMIS and SHULL (13). The reducing sugar yields were not greater than were obtained by hot water extraction alone. Microchemical tests for starch on fresh and preserved maize tissue at various mid- and late-season stages have failed uniformly to give positive reactions although scattered starch grains have been observed in the base of young plants. Fresh leaves from defruited plants have on occasion shown a faint lavender color of amyloextrin, but other materials have stained brown with iodine. Our results modify the conclusions of COLIN and DE CUGNAC (2), who classify maize as storing sucrose rather than starch, to sucrose plus two dextrin-like fractions, but little or no true starch in the vegetative tissues of maize.

The reducing substances removed by boiling 1 + 20 hydrochloric acid in a standard time, usually two hours, are frequently reported as "starch." Since our tests generally showed no starch in the vegetative maize tissues at any stage of development, the term is a misnomer when applied to this plant. The use of acid hydrolysis alone is open also to the serious objection that it does not differentiate between the reserve and structural polysaccharides. In our material, where starch was absent and saliva removed no more material than did hot water, we used acid hydrolysis as a measure of the readily hydrolyzable, hemi-reserve, or more soluble structural substances, present in the dextrin-free residue. Acid hydrolysis is an empirical reaction whose yield depends upon acid strength, time, temperature, and fineness of the sample as well as upon the chemical composition of the substrate.

Data are presented in table II which show that finely ground maize leaf residues contain a large, readily hydrolyzable fraction. A point of stability

TABLE II

ACID HYDROLYSIS WITH 1 + 20 HYDROCHLORIC ACID UNDER REFLUX ON RESIDUES  
FROM ALPHA AND BETA POLYSACCHARIDE EXTRACTIONS

PERIOD	TOTAL TIME	GLUCOSE VALUES* FOR PERIOD	TOTAL GLUCOSE* VALUES
30 min. ....	30 min.	55.80	55.80
1 hr. ....	1.5 hr.	4.50	60.30
1.5 " ....	3 "	3.50	63.80
2 " ....	5 "	1.50	65.30
2 " ....	7 "	1.45	66.75
2 " ....	9 "	1.20	67.95
2 " ....	11 "	1.30	69.25
8 " ....	19 "	2.30	71.55
8 " ....	27 "	2.00	73.55

\* Mg. glucose equivalent per 50 ml. of uniform aliquots.

in the hydrolysis curve was not reached, however, until the treatment had been continued for three hours. Unpublished data obtained in this laboratory by J. C. ELDREDGE show that one hour at 120° C. (autoclave at 15 pounds pressure) is equivalent to refluxing for 4 hours on a boiling water bath in the hydrolysis of maize tissues. The autoclaving method was chosen for the routine analyses because of its greater speed and convenience.

In addition to the three fractions, alpha, beta, and acid hydrolyzable, materials forming reducing substances were extracted by both hot and cold water and precipitated by neutral lead clearing. The losses from clearing shown in table I were approximately 26 per cent. of the alpha- and 31 per cent. of the beta-extraction.

#### PROPERTIES OF THE POLYSACCHARIDE FRACTIONS

**HYDROLYSIS REACTIONS.**—A 5-gm. sample of powdered leaf residue was extracted with cold 10 per cent. alcohol and the extract cleared with neutral lead acetate and filtered. Microscopic examination showed that the clearing precipitate was amorphous and of jelly-like consistency; it gave no color reaction with I-KI, and was filtered out with difficulty. Hydrolysis of the clearing precipitate with 1 + 20 hydrochloric acid at 100° C. for two hours gave slightly less than 100 per cent. recovery of the loss in reducing substances as a result of clearing. Hydrolysis at 120° C. in the autoclave, however, almost completely destroyed the reducing power of the lead-containing material.

The residue from the 10 per cent. alcohol was extracted with hot water, and the two polysaccharide extracts were used for a comparison of the effects of concentration of acid, and of time and method of hydrolysis. The maximum yield of glucose was obtained with 1 + 20 hydrochloric acid, whether hydrolyzed at 100° C. or 120° C. The difference in values by the two methods was small (fig. 1), and hydrolyzing at 120° C. had the advantage that a larger number of samples could be handled more conveniently and in considerably less time. The hydrolysis was not complete even after 4 hr. with 1 + 40 hydrochloric acid at 100° C., while longer than 2 hr. at 120° C. caused a slight destruction of reducing substances.

Hydrolysis of the alpha fraction with 1 + 20 hydrochloric acid at 100° C. for 30 min. yielded about 50 per cent. of the reducing power obtained in the same length of time at 120° C. The comparative yield for the beta fraction, when treated similarly, was considerably less, amounting to about 20 per cent.

Long continued hydrolysis with 1 + 20 hydrochloric acid at 120° C. resulted in destruction of the reducing substances from both the alpha and beta fractions. The beta fraction, however, was not as readily destroyed by the more drastic hydrolysis as was the alpha fraction. In their other characteristics the hydrolysis curves of the beta fraction were comparable to those for the alpha fraction shown in figure 1.



The method commonly used to determine the acid-hydrolyzable material in the starch- and dextrin-free residue is to reflux it for 2.5 hr. with 1 + 20 hydrochloric acid. This method does not give a distinct end point although the values obtained are comparable. Further treatment shows that more reducing substances are obtained with additional hydrolysis. In an accumulative 40-hr. hydrolysis treatment, 75 per cent. of the total reducing substances obtained was extracted in the first 90 min. of hydrolysis. It is suggested that the reducing substances produced after prolonged hydrolysis are the result of the gradual digestion of the less readily hydrolyzed materials

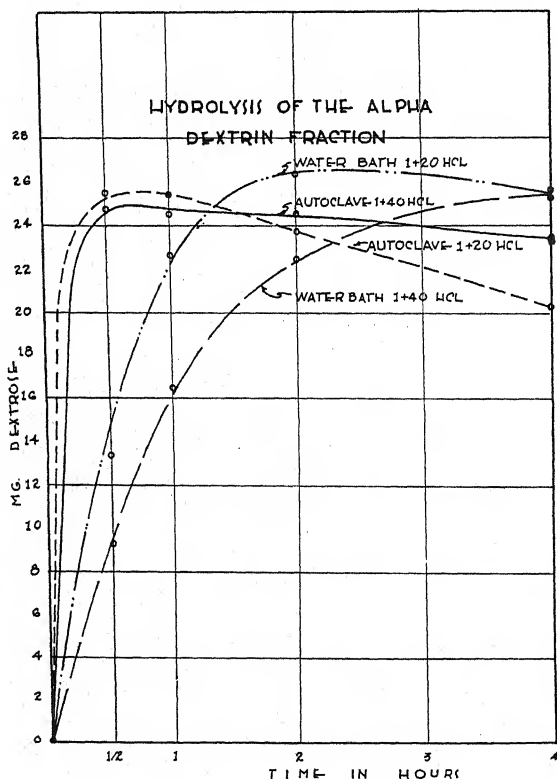


FIG. 1. Hydrolysis curves for varying treatments of alpha extract from maize leaves.

including cellulose. There was, however, a distinct line of demarcation separating the readily and more slowly hydrolyzed polysaccharide fractions, comparable to the endpoints shown in the extraction of the alpha and beta dextrin fractions. The fraction is considered to be that material removed in the first 3 or 4 extractions for the alpha and beta fractions and in the first one to two hours for the acid hydrolyzable materials. Further treatment yields more reducing substances with any of the extractions, but this yield

appears to be caused by the hydrolysis of the more complex underlying materials.

The hydrolyzed materials from each of the three methods of extraction were tested for fructose by the JACKSON and MATHEWS (5) modification of NYN's selective method for the estimation of levulose to determine whether levulosans had been present in the extract before hydrolysis. Negative results were obtained in all cases.

OSAZONE REACTIONS.—A composite sample of the leaf material was extracted with 10 per cent. alcohol, the extract concentrated by evaporation, cleared with neutral lead acetate, and the excess lead removed with sodium oxalate. Sufficient 95 per cent. alcohol was added to the cleared solution to precipitate the dextrin-like material of the alpha fraction. A white, amorphous substance was obtained which gave a reddish brown color with I-KI and was soluble in cold water.

Acid-hydrolyzed portions of a water solution of the precipitate yielded phenyl-osazones when treated with phenyl hydrazine hydrochloride and sodium acetate (1). Since dextrin is assumed to be hydrolyzed to glucose, glucosazones should have been formed. The osazones which were formed, however, crystallized out only upon cooling and more closely resembled malt-osazones than glucosazones in structure.

NOYES *et al.* (15) have reported that glucose is not altered in reducing power by treatment with 10 per cent. hydrochloric acid. It does not follow, however, that glucose was not altered in some way since darkening of the solution after prolonged heating indicated some alteration. In order to determine whether or not the substances produced by acid hydrolysis of the precipitate might be altered by the acid treatment, and as a consequence alter the structure of the osazone formed, comparisons were made of the osazones of the alpha-dextrin fraction and c.p. dextrin.

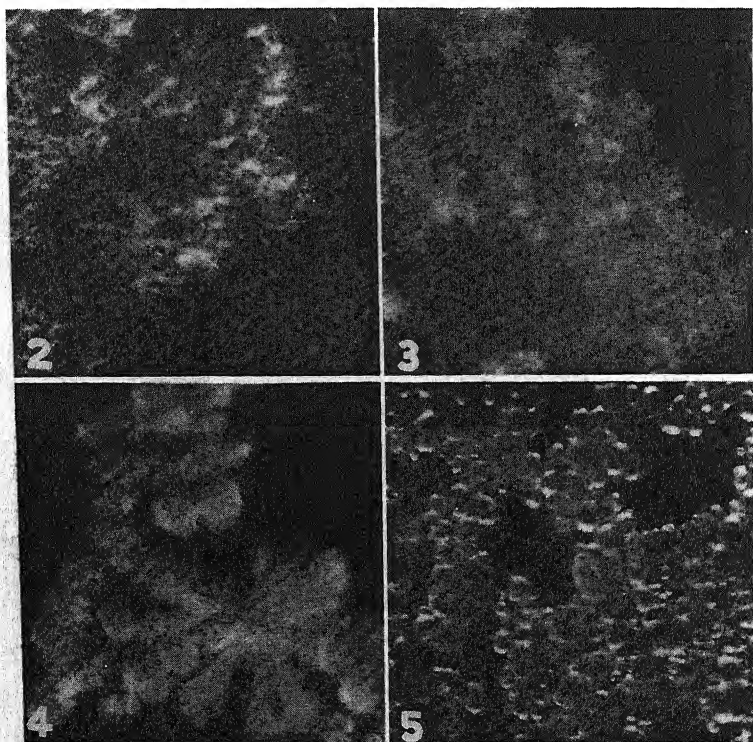
One liter of a 0.10 per cent. solution each of alpha-dextrin and of c.p. dextrin was prepared. One hundred-ml. aliquots of the two solutions were hydrolyzed with 1 + 20 HCl under varying periods of time and temperature as follows:

- |                       |                          |
|-----------------------|--------------------------|
| 1. 30 minutes at 100° | 5. 30 minutes at 120° C. |
| 2. 1 hour " "         | 6. 1 hour " "            |
| 3. 2 hours " "        | 7. 2 hours " "           |
| 4. 4 hours " "        | 8. 4 hours " "           |

Figures 2 to 5 show clearly increasing degrees of alpha extract hydrolysis. The phenyl hydrazine derivatives shown in figure 2 following 30 min. hydrolysis at 100° C. were very small, star-like, crystalline bodies with many amorphous particles distributed throughout the field. Hydrolysis for one hour increased the number and produced more uniformity in the crystals. Numerous sharp yellow needles were formed although they were too small

to resemble glucosazone fragments produced by any hydrolysis treatment performed. Further hydrolysis for two and four hours at 100° C. yielded substances which formed osazones of the type shown in figure 3.

The best crystal formation was obtained with solutions hydrolyzed 30 min. at 120° C. These osazones more closely resembled those formed by glucose than any of the other treatments. Hydrolyzing longer than one hour at



FIGS. 2-5. Phenyl osazones from alpha extract hydrolyzed in 1 + 20 HCl.

Fig. 2. Heated 30 min. at 100° C.

Fig. 3. Heated 4 hr. at 100° C.

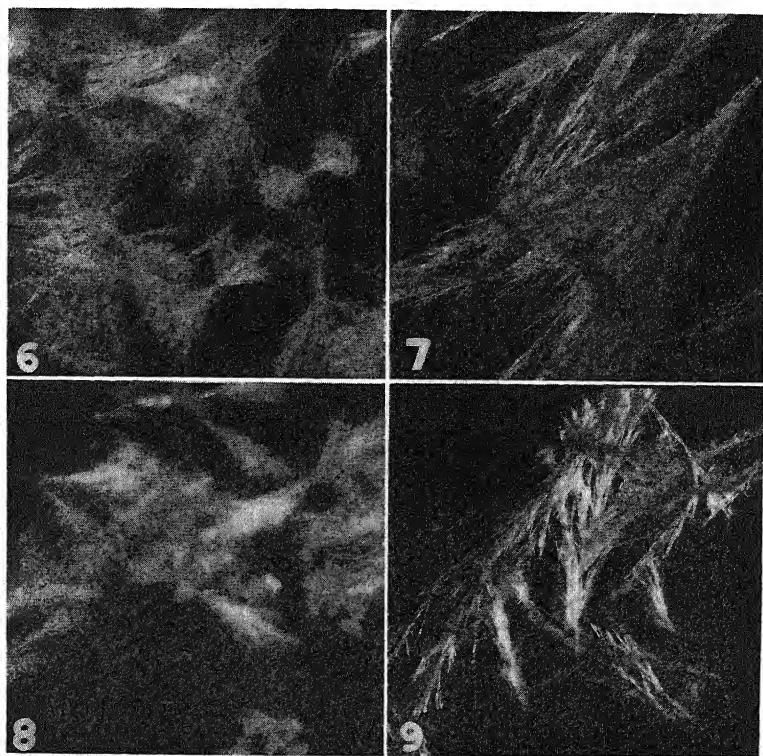
Fig. 4. Heated 30 min. at 120° C.

Fig. 5. Heated 4 hr. at 120° C.

120° C. seemed to bring about, in this case also, a change in the products of hydrolysis and occasioned the formation of what appeared to be isomaltose.

Comparing figures 6 to 9 with figures 2 to 5 it will be observed that the glucosazones formed from dextrin were only slightly altered by the different hydrolysis treatments. It is significant that the crystals obtained from all of the dextrin solutions crystallized out in hot water after 20 to 30 min. heating, while those from the alpha extract of maize crystallized only after

cooling. This character suggests maltose or isomaltose. It does not, however, seem probable that glucose formed by gentle hydrolysis of the alpha fraction should immediately be polymerized with the formation of isomaltose, while the same treatment had no such effect upon pure dextrin. The behavior of the osazones formed by the products of hydrolysis from the alpha extract, together with their structural types show rather definitely that the material



FIGS. 6-9. Phenyl osazones from c.p. dextrine hydrolyzed in 1+20 HCl.

Fig. 6. Heated 30 min. at 100° C.

Fig. 7. Heated 4 hr. at 100° C.

Fig. 8. Heated 30 min. at 120° C.

Fig. 9. Heated 4 hr. at 120° C.

extracted from corn leaf tissue by 10 per cent. alcohol differs from true dextrin in unknown respects.

Although the exact nature of the substance has not been determined it appears that: (a) upon hydrolysis, reducing substances are produced which are not glucose; or (b) the presence of impure gums alters the hydrolytic products in such a fashion as to change their reaction with phenylhydrazine, masking their identity.

Phenyl osazones produced from the hot-water soluble (beta) extract closely resembled glucosazones but a mixture of osazones was observed suggesting the presence of materials other than glucosans.

#### VARIATIONS IN THE POLYSACCHARIDE FRACTIONS

DIURNAL VARIATIONS.—LOOMIS (10) has reported that sucrose shows the most important diurnal variations among sugars of the leaf and stem of maize. In our experiments sucrose in the leaves increased 1.5 per cent. to four times

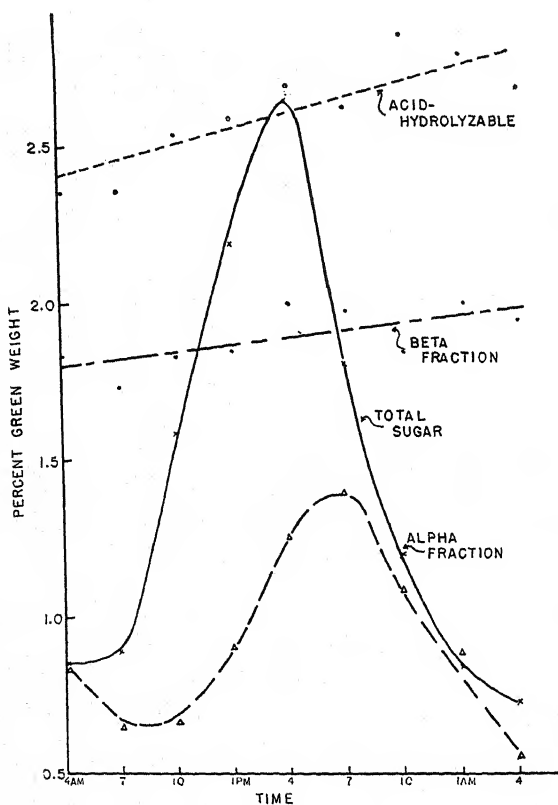


Fig. 10. Diurnal variations in the carbohydrates of maize leaves. July 19–20, 1932.

its original value between 4 A.M. and 4 P.M. Sucrose in the stem increased from a low of 0.27 per cent. at 7 A.M. to 0.90 per cent. at 7 P.M. on July 15, the maximum showing a lag of three hours in comparison with the leaf maximum. Polysaccharide analyses were made on series of samples collected July 19, 1932 and July 15, 1933. They showed variations of nearly 100 per cent. in the alpha fraction in both seasons. Neither the beta nor the acid-hydrolyzable fraction showed significant diurnal variations in the 1932 analyses. In

1933, however, when these two fractions were determined together, they showed as much variation as did the alpha fraction. Data for the two series are shown in tables III and IV and in figures 10 and 11, where the total sugar curves are included for comparison. Total sugar variations, as stated above, were mainly attributable to sucrose. Cold-water soluble polysaccharides are shown to exhibit diurnal fluctuations in the leaves of silking maize with a tendency for the maximum to follow the maximum of total sugars by about three hours. The differences in the variation of the beta fraction in the two years are not understood. In one series of ground samples left exposed to laboratory air, there were important shifts in the polysaccharide solubilities after several weeks. Changes of this type may have entered into our comparisons although all of the samples were held in sealed containers.

TABLE III

DIURNAL VARIATIONS IN THE CARBOHYDRATES OF MAIZE LEAVES, JULY 19, 1932  
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	TOTAL SUGARS	ALPHA- EXTRACT	BETA- EXTRACT	ACID- HYDRO- LYZABLE	TOTAL POLYSAC- CHARIDES
	%	%	%	%	%
4 A.M. ....	0.84	1.01	1.83	2.38	5.22
7 A.M. ....	0.92	0.66	1.73	2.37	4.76
10 A.M. ....	1.58	0.67	1.82	2.54	5.03
1 P.M. ....	2.19	0.91	1.86	2.60	5.37
4 P.M. ....	2.65	1.28	2.01	2.68	5.97
7 P.M. ....	1.82	1.40	1.97	2.63	6.00
10 P.M. ....	1.19	1.09	1.84	2.88	5.81
1 A.M. ....	0.83	0.89	2.00	2.78	5.67
4 A.M. ....	0.73	0.54	1.95	2.67	5.16

The polysaccharides of the stems showed no diurnal variation. On the green weight basis, however, they showed a steady seasonal increase in the percentage of total polysaccharides. This increase was shown to be caused by the loss of water from the maturing pith, thus one would conclude from the data that there were no significant fluctuations in the polysaccharides of maize stalks during the periods of study.

#### THE RELATION OF TEMPERATURE TO DIURNAL POLYSACCHARIDE VARIATIONS

SPOEHR (17) reported that the polysaccharide-sugar ratio in cacti is dependent upon at least two factors: temperature, and water content. LOOMIS and EVANS (12) and LOOMIS (8) showed that storage of tubers and corms at temperatures around 33° C. resulted in a reduction of the rest period to one-third of the normal duration, and in a change of polysaccharide reserves to soluble carbohydrates. On the other hand, the rest period was broken and starch was changed to sucrose in tulip bulbs stored at 5° C.

TABLE IV

DIURNAL VARIATIONS IN THE CARBOHYDRATES OF MAIZE LEAVES, JULY 15, 1933  
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	TOTAL SUGARS	ALPHA-EXTRACT	BETA-EXTRACT PLUS ACID-HYDROLYZABLE	TOTAL POLY-SACCHARIDES
	%	%	%	%
4 A.M. ....	0.80	0.65	4.94	5.59
7 A.M. ....	1.17	0.65	4.66	5.31
10 A.M. ....	1.57	0.74	4.95	5.69
1 P.M. ....	2.11	0.84	5.20	6.04
4 P.M. ....	2.33	1.32	5.37	6.69
7 P.M. ....	1.78	1.28	5.11	6.40
10 P.M. ....	1.39	0.99	4.94	5.93
1 A.M. ....	1.19	1.03	5.00	6.03
4 A.M. ....	1.15	0.85	4.61	5.46

CURTIS (3) in his studies on solute translocation in plants found that chilling bean petioles to temperatures between 1° and 4° or 6° C. greatly retarded, or stopped, the removal of carbohydrates from leaf blades but that

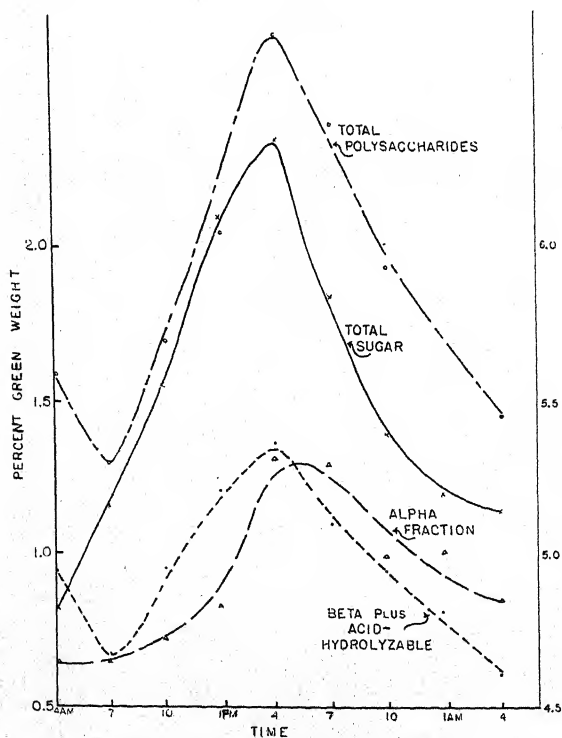


FIG. 11. Diurnal variations in the carbohydrates of maize leaves. July 15-16, 1933.

translocation was not stopped by chilling from 25° to 6° or 8° C. In a later paper CURTIS and HERTY (4) found that chilling bean petioles to temperatures between 0.5° and 4.5° C. greatly retarded the removal of carbohydrates from leaf blades but that translocation was not completely stopped by chilling for 17 to 20 hours.

The data of figures 10 and 11 show that in the period from 4 to 7 P.M. while the sugar value was rapidly declining, the total polysaccharide value still showed an upward trend in the leaves. This accumulation of total polysaccharides was accounted for by an increase in the alpha extract. The total sugar content increased in the morning with increased temperature and solar radiation but the maximum sugar content occurred three hours later than the maximum temperature which was reached at 1 P.M. Temperatures seemed to be more closely correlated with diurnal accumulation of sugars than of polysaccharides unless the late afternoon shift to polysaccharides can be interpreted as being the result of falling temperature.

In an investigation of the relationship between temperature and the polysaccharide-sugar balance, experiments were performed upon cut plants held in water and stored at different temperatures. Very little change in carbohydrate content was shown by the different treatments in either total sugars or total polysaccharides.

Samples held at 11° C. were slightly higher in carbohydrates than were those held at 28° C. indicating the utilization of more carbohydrate material in respiration at the higher temperature. There was, however, no evidence of a shift from polysaccharide to sugar or sugar to polysaccharide as a result of the temperature treatment.

#### SEASONAL VARIATIONS

Our data showed very little change in the percentage of the alpha fraction in either the leaves or stems of maturing maize plants. The acid-hydrolyzable fraction, however, being derived largely from products of differentiation in the aging cells, showed more accumulation. The greatest increase in the leaves was one per cent., calculated on the green weight basis, while the stem polysaccharides increased as much as 4 per cent. from July to August, 1933. It must be kept in mind, however, that the stems become pithy as a result of the loss of water so that their green weight per unit volume decreases rapidly.

When the carbohydrates were calculated as the percentage of residual dry weight, that is, of the dry weight of the tissue after the soluble carbohydrates and the polysaccharides are removed, somewhat different results were obtained. The alpha fraction of both the leaf and stem tissues showed the same general trend while the total polysaccharides of the leaves decreased in the 1932 experiments, and the percentage increase in the 1933 series was lower



than when calculated on the green weight basis. Total stem polysaccharides on August 29, 1932 were about 400 per cent. of their value on July 19 on the green weight basis; when estimated on the residual dry weight basis, however, the August 29 value was only 104 per cent. of that obtained on July 19. The variation in the carbohydrate content of this pithy tissue with its rapidly changing water content should be more accurately expressed on the basis of residual dry weight (total dry weight less carbohydrates) than on the basis of either fresh weight or total dry weight. If this viewpoint is correct we may conclude that hemi-cellulose materials are not deposited in the vegetative cells of maturing corn plants; particularly not in the pith cells of stalks.

#### EFFECT OF DEFRUITING UPON THE POLYSACCHARIDES OF MAIZE

WILLAMAN *et al.* (19) and LOOMIS (11) have shown that removing the ear caused sugar to accumulate in maize plants. There was likewise a marked seasonal accumulation of sugar in our plants from which the fruits had been removed. In spite of this accumulation of sugar, relatively little accumulation of the alpha extract or total polysaccharides was observed in the stems of defruited plants. Only the alpha fraction in the leaves showed an accumulation which amounted to about double the quantity found in the check leaf tissue.

A comparison of the different polysaccharide fractions in the leaves and stems of fruiting maize plants in the soft dough stage, and of plants from which the ears were removed at silking time was made. The data are somewhat meager as they represent only 4 collections taken rather late in the season and following an abnormally low temperature during the previous night. They should, however, be adequate to show whether the surplus carbohydrates of the defruited plants are stored in the stalk. Rather surprisingly there is no marked evidence of such storage, neither the diurnal variations nor the actual percentage values of the polysaccharides in the defruited plants being strikingly different from those of the check plants. LOOMIS (11) has reported that the stalks of defruited maize plants were frequently heavier than normal although the total weight of stalk and grain in the checks was much greater than that of the defruited plant. He reported, also, sucrose accumulations of 3 or more per cent. of the green weight in the defruited stalk above the values in control stalks. Our analyses, shown in table V, indicate increases of less than one per cent. in alpha polysaccharides, with much smaller increases in the other two fractions. A well developed ear of maize weighs as much as the vegetative plant and the observed accumulations of polysaccharides in the stalk must be contrasted with the normally heavy storage of starch in the grain. The conclusion that the stalks of defruited maize do not replace the ear as storage organs seems to be justified.

TABLE V  
POLYSACCHARIDE ANALYSES OF CONTROL AND DEFRUITED MAIZE PLANTS  
(PERCENTAGE ON BASIS OF GREEN WEIGHT)

TIME	ALPHA FRACTION		BETA FRACTION		ACID HYDROLYZABLE	
	CHECK	DEFRUITED	CHECK	DEFRUITED	CHECK	DEFRUITED
	%	%	%	%	%	%
LEAVES						
4 A.M. ....	1.11	2.65	0.54	0.74	5.54	4.44
10 A.M. ....	1.23	1.83	0.41	0.68	5.47	5.44
4 P.M. ....	1.53	2.47	0.53	0.83	5.54	5.59
10 P.M. ....	2.02	2.35	0.63	0.71	5.71	5.92
STEMS						
4 A.M. ....	0.73	1.11	0.25	0.28	5.20	5.60
10 A.M. ....	0.51	1.36	0.23	0.35	4.92	5.17
4 P.M. ....	0.41	1.17	0.19	0.31	5.78	5.82
10 P.M. ....	0.57	1.48	0.29	0.39	5.36	6.01

### Summary

1. The polysaccharides of vegetative maize tissues have been separated into 4 fractions on the basis of solubility. The first fraction is readily soluble in cold 10 per cent. alcohol and may constitute one or two per cent. of the green weight of the tissue. The second fraction is slowly soluble in boiling water; the third fraction is quickly removed by boiling 1+20 HCl and the residual fraction, mainly cellulose, is resistant to hot 1+20 acid. A fifth fraction may have been present in the neutral lead acetate precipitates from the water extracts.

2. The solubility reactions and hydrolysis curves of the two cleared, water-extractable materials, designated as the alpha and beta fractions or extracts, corresponded to those of dextrans of medium and high molecular weight respectively. Copper reduction methods indicated that the hydrolysates were free of fructose. Osazone reactions of the alpha hydrolysate indicated impure maltose, while those of the beta fraction indicated impure glucose. Although more work is needed to characterize these polysaccharides, they appear to be mixtures of dextrin with other water-soluble gums.

3. The acid-hydrolyzable fraction was commonly larger than the total of the two water-soluble fractions. A reasonably sharp endpoint for the hydrolysis was obtained after 3 hours at 100° C. or 1 hour at 120° C. with 1+20 HCl. This fraction showed no significant daily or seasonal variations after the plants reached the silking stage, except as the results of the pith analyses affected by the loss of water from the pith of maturing cornstalks. When these data were calculated to a sugar- and dextrin-free dry weight basis the apparent late season increases disappeared.

4. No starch has been found in any vegetative tissue of maize except for

a few grains near the bundles of stunted seedlings. Starch does not appear to be a constituent of the growing or maturing plant.

5. The alpha-polysaccharide fraction showed diurnal fluctuations in the leaves which were nearly half as great as the sugar fluctuations and lagged approximately 3 hours behind them. Beta-fraction variations were inconsistent and may have been caused by secondary changes within the ground sample. Acid-hydrolyzable and residual materials showed a seasonal accumulation on the green weight basis which disappeared when calculations were based on residual dry weights.

6. Removing the ear at silking resulted in alpha-fraction accumulations of less than one per cent. of the green weight and in smaller accumulations of the other fractions. The maize stalk is not an effective storage organ.

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# A STUDY OF DEVELOPMENTAL CHANGES IN COMPOSITION OF THE MACADAMIA<sup>1</sup>

WINSTON W. JONES

(WITH SIXTEEN FIGURES)

## Introduction

In recent years the macadamia [*Macadamia ternifolia* F. von Mueller, var. *integrifolia* (Maiden and Betcher) Maiden] has assumed increasing commercial importance in the Hawaiian Islands. The Hawaii Agricultural Experiment Station has placed emphasis on establishing varieties and developing improved cultural practices, and toward the latter end it has become desirable to secure knowledge concerning the normal physiological development of this interesting fruit. The present paper sets forth results of a study on the changes in size, weight, and chemical composition of the macadamia fruit from flowering to maturity.

Preliminary work (7) has shown that this fruit requires from 215 to 230 days after flowering to reach maturity, and that the mature dry embryo contains about 75 per cent. oil. Very few studies have been reported on the physiological development of perennial oil-seed fruits since THOR and SMITH (11) reported on the pecan. Studies on oil-seeds have been largely confined to quick growing crops such as cotton (4), flax (2), rape, hemp, poppy, and sunflower (5). CHEEL and MORRISON (1) describe some characteristics of macadamia oil, but, other than a preliminary paper by the writer (7), no physiological account of fruit development in macadamia has thus far appeared.

## Materials and methods

Fruits were collected at five intervals from flowering to maturity from five well-bearing seedling trees, selected on a basis of apparent uniformity in fruiting characteristics, in a commercial orchard near Honolulu. Figure 1 shows the approximate size of the macadamia at the various stages of maturity selected for analyses.

The first samples were taken approximately 90 days after flowering; before this time the embryos were too small to analyze. One hundred fruits from each tree were used for the first two samplings and fifty for the later samples. The sample from each tree was weighed and the average weight of the fruit determined. Each fruit was sliced through the middle; the diameter of the unhusked nut, the thickness of the shell, and the diameter of the embryo were measured. The material was then separated into husks,

<sup>1</sup> Published with the permission of the Director, Hawaii Agricultural Experiment Station.

shells, and embryos<sup>2</sup> and the fresh weights of the fractions from each tree obtained. Dry weights were obtained after the fractions had been held at 100° C. for 2 to 3 hours and under vacuum at 80° C. for 48 hours.

Preliminary work (7) has shown that the husk and shell change in composition very little during the growth and maturity of the macadamia; thus in the present study chemical analyses were made of the kernel (embryo) only.

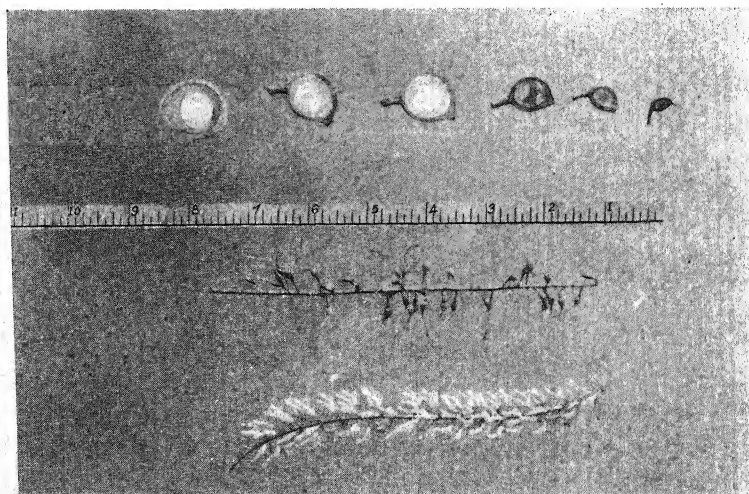


FIG. 1. Lower, macadamia flower cluster; middle, cluster of young nuts; upper, cross section of fruit showing stages at which samples were taken (fruit at extreme right too small for sampling).

### OIL

After the first two samples the embryos could not be ground without loss of oil, so the samples were chopped with a sharp knife. The finely chopped material was re-dried in a vacuum oven at 80° C. for 24 hours, and 2.5-gm. aliquots were weighed into alundum cups and extracted for 6 hours with anhydrous alcohol-free ether in a continuous drip extractor. The ether was then removed, and the extract dried, weighed, and reported as oil.

### SUGARS

The oil-free residue was extracted with 80 per cent. alcohol. An aliquot of the extract was cleared with neutral lead acetate and potassium oxalate; reducing sugars were determined by the method of STILES, PETERSON, and FRED (10). Sucrose was obtained by the same method after inversion with invertase.

<sup>2</sup> The so-called nut of the macadamia is a true seed; the shell is composed of the outer integument while the edible portion, or kernel, is the embryo (6).

## NITROGEN

Soluble nitrogen was determined on aliquots of the alcoholic extract by the reduced-iron method of PUCHER, LEAVENWORTH, and VICKERY (9), adapted to the micro-Kjeldahl method described by PREGL (8). Insoluble nitrogen was determined on the residue from the alcoholic extraction by the micro-Kjeldahl method of PREGL. At all times the embryo gave a negative test with diphenylamine, indicating the complete absence of nitrate from this organ.

## Presentation and discussion of data

The environmental conditions prevailing during the growth of the fruit, including the weekly average day and night temperatures, the weekly average of sunlight intensity, and the weekly rainfall, are shown in figure 2.

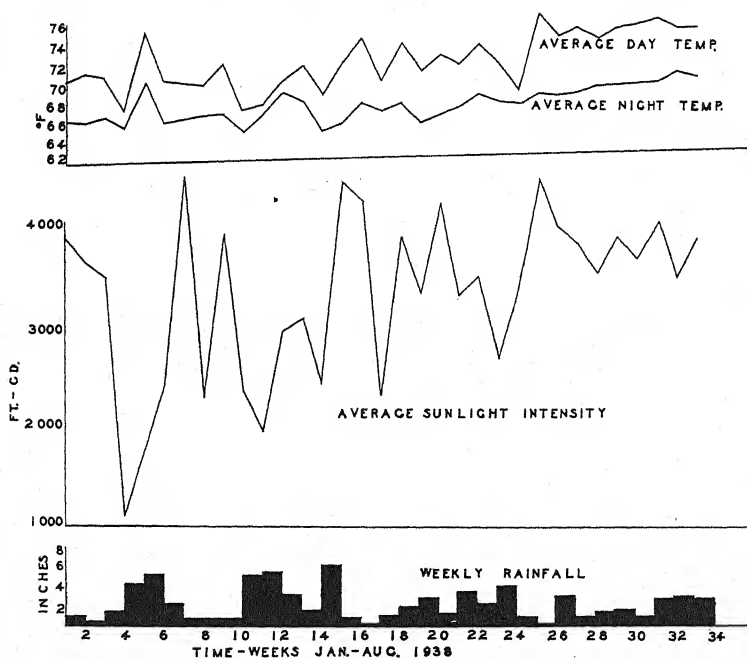


FIG. 2. Rainfall, sunlight, and temperature at Nutridge, Oahu.

The accumulation of dry matter in the macadamia fruit and its parts is shown in tables I and II and figures 3 and 4. The remainder of the analytical data are presented graphically in figures 5 to 16. The graphs show the curves for the individual seedling trees so that the variations are more, in some cases, than if a single clone had been available or if the fruit from several trees had contributed to a single sample. In most instances, however, the trends of development are similar.



TABLE I

SIZE CHANGES IN THE MACADAMIA FRUIT DURING DEVELOPMENT

DATE	DAYS AFTER FLOWER- ING	TREE NO.	AVERAGE FRESH WEIGHT OF WHOLE FRUIT	AVERAGE DIAMETER OF UN- HUSKED NUT	AVERAGE DIAMETER OF EMBRYO	AVERAGE THICK- NESS OF SHELL	CONDITION OF SHELL
			<i>gm.</i>	<i>mm.</i>	<i>mm.</i>	<i>mm.</i>	
4-13-38	90	2	3.02	17.92	9.08	2.32	Soft
5- 4-38	111		8.64	24.56	16.68	2.10	"
	111		9.30	25.44	16.88	2.06	Hard—white
5-19-38	126		10.24	26.09	16.86	2.29	" —light brown
7-13-38	185		11.32	26.98	17.38	1.83	" —brown
8-22-38	215		12.37	27.76	17.54	2.15	" —dark brown —nuts mature
4-13-38	90	4	3.61	17.44	9.64	2.12	Soft
5- 4-38	111		7.76	23.68	15.92	2.24	"
	111		8.58	24.44	16.00	2.14	Hard—white
5-19-38	126		10.70	26.91	17.95	2.51	" —light brown
7-13-38	185		12.42	28.24	17.76	2.36	" —brown
8-22-38	215		13.52	28.91	18.20	2.74	" —dark brown —nuts mature
4-14-38	90	6	7.33	24.02	15.92	1.97	Soft
5- 5-38	111		12.92	27.48	18.42	2.13	Hard—white
5-20-38	126		10.29	26.26	17.60	2.08	" —light brown
7-14-38	185		13.72	28.68	18.08	2.12	" —brown
8-23-38	215		13.51	28.70	18.18	2.17	" —dark brown —nuts mature
4-14-38	90	7	6.60	22.14	14.24	2.09	Soft
5- 5-38	111		13.08	28.38	18.42	2.61	Hard—white
5-20-38	126		14.14	28.94	19.00	2.43	" —light brown
7-14-38	185		17.10	30.70	19.62	2.28	" —brown
8-23-38	215		17.26	30.82	20.00	2.32	" —dark brown —nuts mature
4-14-38	90	9	4.32	19.28	11.00	1.75	Soft
5- 5-38	111		13.32	28.70	18.94	2.15	Hard—white
5-20-38	126		13.92	28.78	18.88	2.22	" —light brown
7-14-38	185		14.70	29.64	18.90	1.86	" —brown
8-23-38	215		16.00	30.22	19.76	1.93	" —dark brown —nuts mature

## GROWTH OF THE MACADAMIA FRUIT

The data of table I show the changes with time in size and fresh weight of the fruit. In no case had the shells hardened in the 90-day sample; at 111 days it will be noted that all the fruit from trees 2 and 4, although of the same age, were not at the same stage of development. The shell was soft on a part of the fruit and on the remainder the shells had hardened; all of the fruit from trees 6, 7, and 9 had hard shells at 111 days. From table I it is apparent that by the time the shell hardened the fruit had reached approximately 70 per cent. or more of its final fresh weight. After the shell hardened the diameter of the embryo and the thickness of the shell increased only slightly.

TABLE II  
DRY WEIGHT CHANGES IN THE MACADAMIA FRUIT

DATE	DAYS AFTER FULL FLOWER	TREE NO.	AVERAGE DRY WEIGHT PER FRUIT OF				RELATION TO TOTAL DRY WEIGHT OF		
			WHOLE FRUIT	HUSK	SHELL	EMBRYO	HUSK	SHELL	EMBRYO
			<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	<i>gm.</i>	%	%	%
4-13-38	90	2	0.59	0.46	0.12	0.01	77.6	20.2	2.2
5- 4-38	111		1.32	0.95	0.48	0.09	62.3	31.7	6.0
	111		2.47	1.06	1.40	0.21	42.9	48.6	8.5
5-19-38	126		3.59	1.17	1.99	0.43	32.6	55.4	12.0
7-13-38	185		5.32	1.58	2.40	1.34	29.7	45.1	25.2
8-22-38	215		6.31	2.00	2.84	1.47	31.7	45.0	23.3
4-13-38	90	4	0.64	0.46	0.15	0.02	72.6	23.8	3.6
5- 4-38	111		1.37	0.80	0.48	0.09	58.4	35.1	6.5
	111		1.49	0.50	0.83	0.16	33.7	55.4	10.8
5-19-38	126		3.32	1.02	1.86	0.44	30.8	55.9	13.3
7-13-38	185		6.37	1.48	3.40	1.49	23.2	53.4	23.4
8-22-38	215		9.38	3.82	3.85	1.71	40.7	41.1	18.2
4-14-38	90	6	1.41	0.92	0.40	0.09	65.4	28.2	6.4
5- 5-38	111		4.28	1.26	2.16	0.86	29.5	50.5	20.0
5-19-38	126		3.87	1.06	1.93	0.88	27.4	49.8	22.8
7-13-38	185		4.48	1.76	0.80	1.92	39.3*	17.9*	42.8*
8-22-38	215		6.59	1.91	2.84	1.84	29.0	43.1	27.9
4-14-38	90	7	0.84	0.52	0.26	0.06	62.0	31.0	7.0
5- 5-38	111		4.96	1.47	2.59	0.90	29.6	52.3	18.1
5-19-38	126		6.08	1.48	3.31	1.29	24.3	54.4	21.3
7-13-38	185		8.68	2.38	4.06	2.24	27.4	46.8	25.8
8-22-38	215		8.89	2.47	4.11	2.31	27.8	46.2	26.0
4-14-38	90	9	0.90	0.69	0.17	0.04	76.8	19.1	4.1
5- 5-38	111		4.08	1.60	1.94	0.54	39.2	47.5	13.3
5-19-38	126		5.40	1.83	2.61	0.96	33.9	48.3	17.8
7-13-38	185		6.21	2.02	2.64	1.55	32.5	42.5	25.0
8-22-38	215		6.90	2.31	2.98	1.61	33.5	43.2	23.3

\* It may be seen from table I that the fruits in this particular sample were small with thin shells, hence the seeming discrepancies in these figures.

The data of table II show the changes in dry weight with time in the macadamia fruit, while the graphs of figures 3 and 4 show the moisture changes. It will be noted from table II that there was an increase in total dry weight of the fruit and its parts from the first to the last sampling date (except for the 4th sample in tree no. 6 in which the sampled nuts were small (see table I). This is in contrast to the total fresh weight shown in table I, in which the increase was slight after the shells became hard and light brown. Figures 3 and 4 indicate a marked decrease in moisture content of the embryo and shell, which accounts for the lack of increase in total fresh weight even though the dry weight is increasing.

#### CHEMICAL CHANGES IN THE MACADAMIA EMBRYO

OIL.—Figures 5 and 6 present the data on oil formation in the macadamia embryo. During the first 90 days after flowering there was very little

oil formation and only slight enlargement of the embryo. Thereafter the embryo began to enlarge, and oil formation was rapid so that 40 days later, or about 130 days after flowering, the oil had reached more than half of the final concentration. During the last 30 days before maturity there was very little increase in the percentage concentration of oil. There is much more variation between the individual trees on the basis of total oil per fruit than

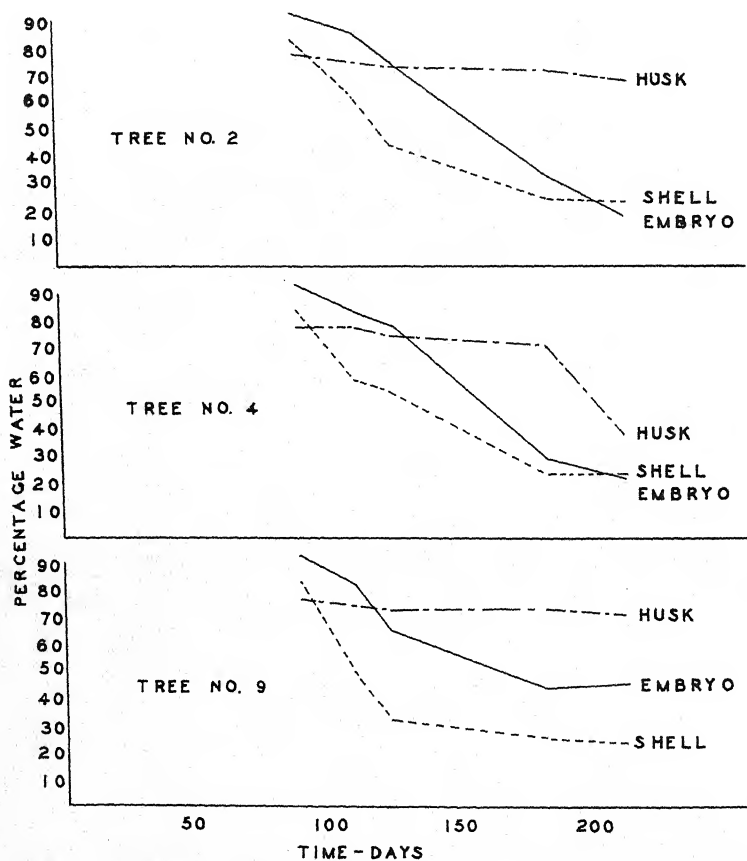


FIG. 3. Moisture changes in the parts of the macadamia fruit, trees 2, 4, 9.

there is on the percentage basis, as seen from a comparison of figures 3, 4 and 5. This difference is attributable to the variations in the size of the fruit.

In three cases the quantity of oil per embryo increased until the time of harvest, while in two cases there were slight drops. In considering an average of the five trees, however, the total oil per embryo showed an almost straight line increase from the beginning of oil formation at 90 days after flowering until maturity 215 days after flowering. This uniform gain is in

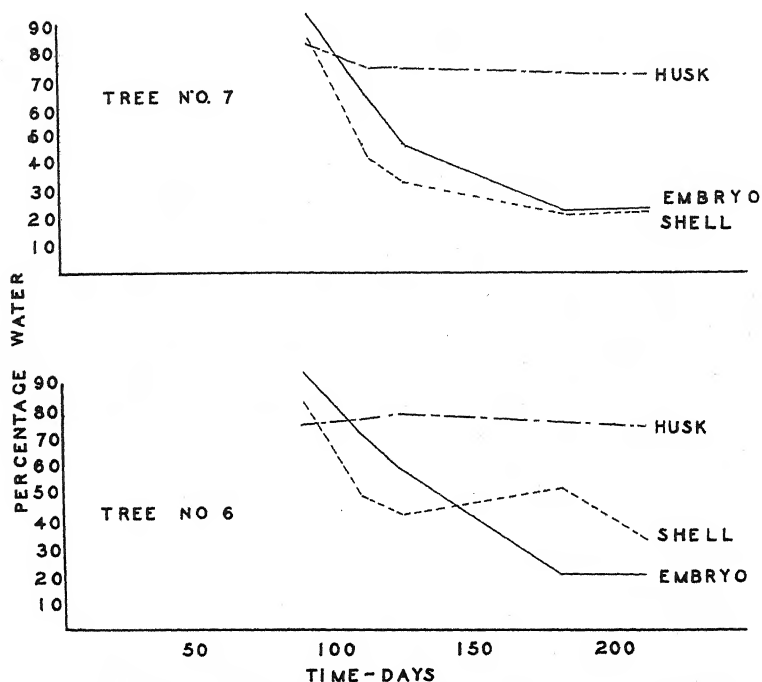


FIG. 4. Moisture changes in the parts of the macadamia fruit, trees 6, 7.

contrast to the pecan (11) which shows no increase in amount of oil per nut during the 25 days preceding harvest. Why the total oil per embryo of two trees should have decreased is not clear, although it is possible that the phenomenon will prove to be related to the beginning of germination of the embryo. Since a part of the fruit for the last sample was harvested from the ground, in the commercial manner, and since the macadamia germinates

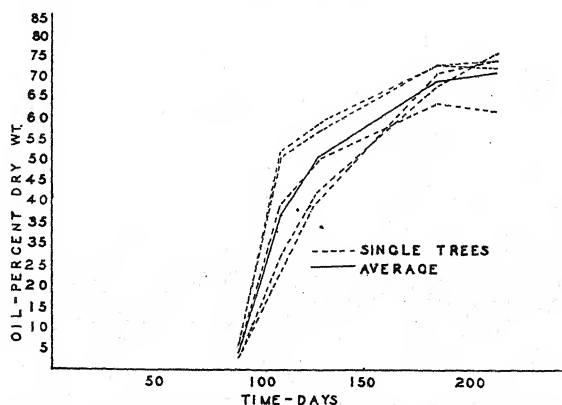


FIG. 5. Changes in oil content during development of the macadamia embryo.

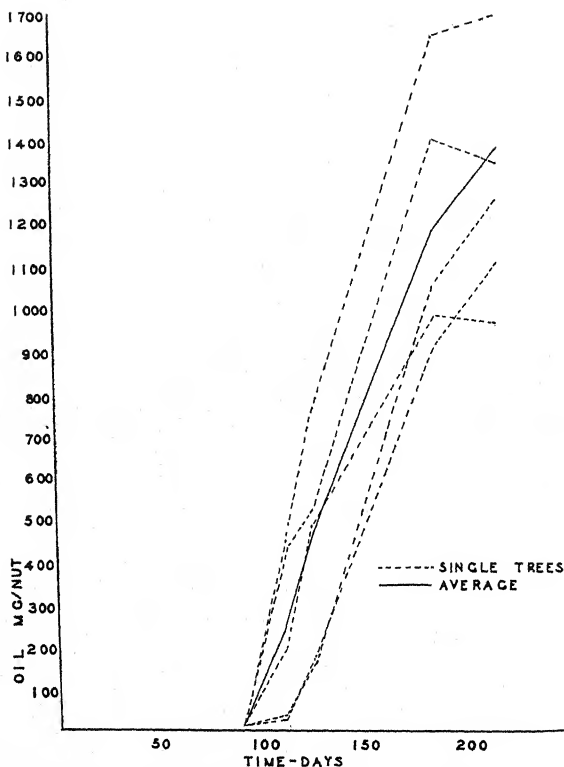


FIG. 6. Changes in oil content during development of macadamia embryo.

readily (in some cases before the nuts drop), some germination may have begun in these two samples although no detectable growth had occurred.

#### OIL-FREE SOLUBLE SOLIDS

Figures 7 and 8 show the changes in oil-free soluble solids of the embryo. On the dry weight percentage basis, there was a rapid decrease from the 90th to the 111th day and then a more gradual decrease until the 215th day, at which time the fruits were considered mature. On the basis of milligrams per embryo, however, the oil-free soluble solids showed an increase until the fourth sampling date, 185 days after flowering, followed by a slight decrease between the 185th and 215th days. The variation between these two figures demonstrates the danger of expressing data on a percentage of dry weight basis, especially when there is a second factor (the rapidly increasing oil in the present case).

#### SUGARS

Figures 9, 10, 11, and 12 show the sugar changes in the macadamia embryo during development. From figure 9 it is noted that the percentage

of reducing sugar fell to a low level at 126 days and remained low until the 185th day; thereafter a slight increase occurred to the last harvest at 215 days. The explanation for this increase in reducing sugar is not clear. As discussed under oil, it might have been caused by the beginning of germination. Another plausible explanation is that, after the rate of formation

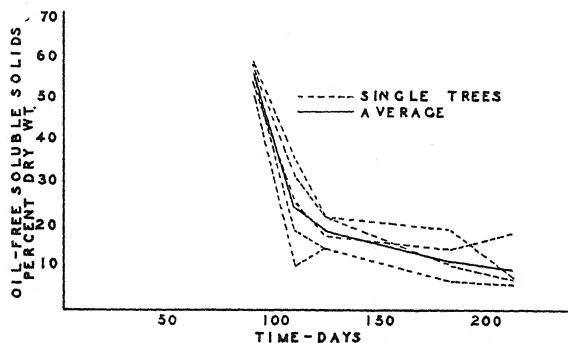


FIG. 7. Oil-free soluble solids.

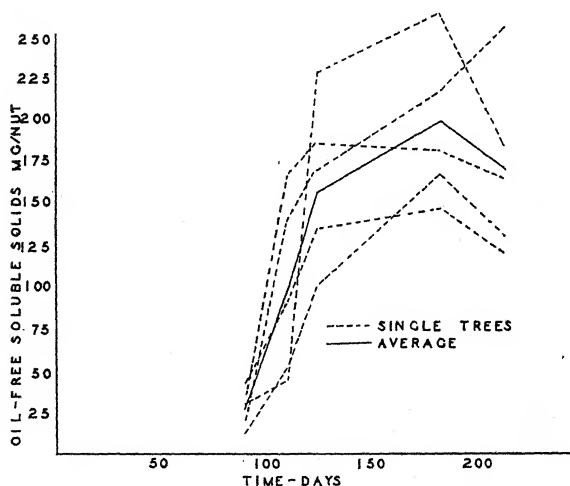


FIG. 8. Oil-free soluble solids.

of oil has decreased, the movement of carbohydrates into the fruit is not retarded proportionally so that reducing sugars accumulate in the embryo. No starch was found in the embryo at any time. Figure 11 shows the quantity of reducing sugar per embryo. The average for the five individual trees shows an increase in total amount up until the 126th day. Beginning at 126 days there was a decrease to the 185th day and then a marked increase to maturity.

Figure 10 shows that sucrose increased, on a percentage basis, until about the 126th day and then gradually decreased to a minimum at maturity. The quantity of sucrose per embryo (fig. 12) also increased until about the 126th

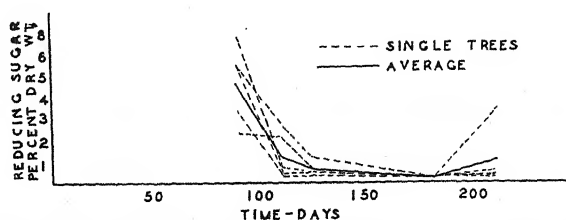


FIG. 9. Changes in reducing sugar content during development of the macadamia nut.

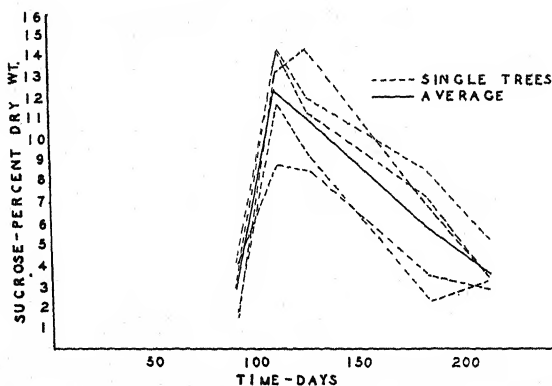


FIG. 10. Changes in sucrose content during development of the macadamia nut.

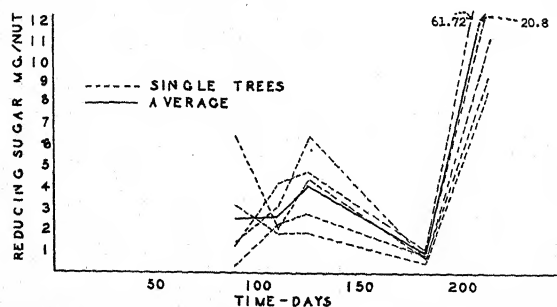


FIG. 11. Changes in reducing sugar content during development of the macadamia nut.

day after flowering, but the total amount remained more or less constant until the 185th day, after which there was a slight decrease to maturity.

#### NITROGEN

Figures 13 to 16 present the data on changes in nitrogen during development of the embryo. Nitrate-free soluble nitrogen maintained a constant

low percentage value after 126 days but on the basis of quantity per embryo it reached a minimum at 111 days and then increased to the 185th day. There was a slight drop between the 185th and 215th days. Insoluble nitrogen (fig. 14), on a percentage basis, reached a maximum at 126 days, de-

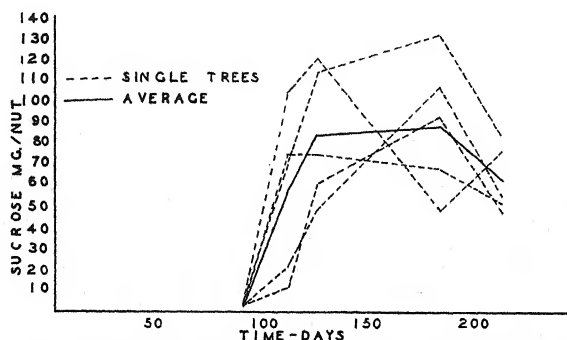


FIG. 12. Changes in sucrose content during development of the macadamia nut.

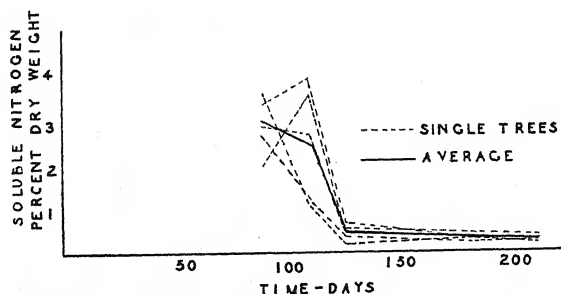


FIG. 13. Changes in soluble nitrogen during development of the macadamia nut.

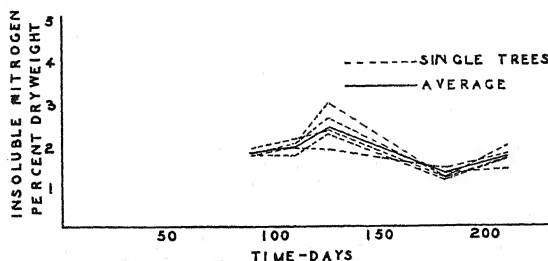


FIG. 14. Changes in insoluble nitrogen during development of the macadamia nut.

creased to the 185th day, then increased slightly to maturity. When the total weight of insoluble nitrogen per embryo (fig. 16) is considered, there was an increase from the first to the last sample. The total amount of nitrogen (insoluble plus soluble) per embryo also increased throughout the development of the fruit.



### Summary

1. A study was made of the physiological development of the macadamia embryo. No chemical analyses are presented for any parts of the fruit other than the embryo, since preliminary work has shown very little change in the husk and shell except during early stages.

2. The development of the fruit is characterized by two distinct periods:

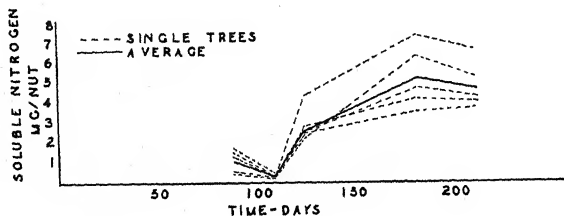


FIG. 15. Changes in soluble nitrogen during development of the macadamia nut.

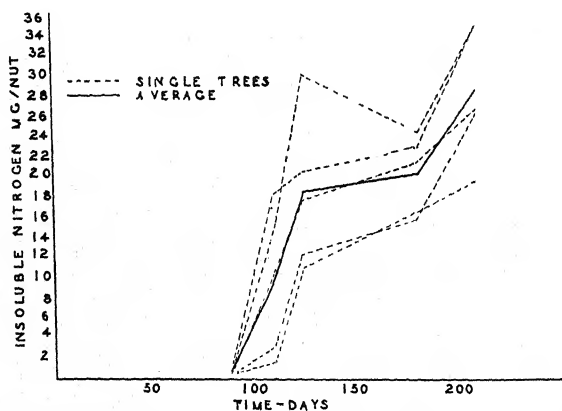


FIG. 16. Changes in insoluble nitrogen during development of the macadamia nut.

the first is from flowering to the end of 90 days, during which very little oil is formed and the embryo does not enlarge sufficiently for analysis; the second is from 90 days to maturity, a period of about 125 days, during which oil is formed and the major expansion of the embryo occurs.

3. The total amount of sugar present increases during the early oil formation but decreases as maturity is attained.

4. Protein synthesis occurs during the same period as oil synthesis.

Thanks are due MR. BRUCE COOIL for assistance in collecting the samples and to MR. HISASHI KUBOTA for a part of the analytical work.

Appreciation is herewith expressed to the Hawaiian Macadamia Nut

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HAWAII AGRICULTURAL EXPERIMENT STATION  
HONOLULU, T. H.

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# THE EFFECT OF HOST NUTRITION ON CONCENTRATION OF TOBACCO-MOSAIC VIRUS

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(WITH ONE FIGURE)

## Introduction

It is well known that variations in the nitrogen nutrition of tobacco plants drastically alter their susceptibility to infection with tobacco-mosaic virus (16) and the rate of spread of virus in the tissues (17). Little attention has been given to the possible effects of nitrogen variations on the virus entity itself. In order to determine whether variations in the nitrogen content of the nutrient solution would bring about changes in the virus concentration<sup>1</sup> within the plant, tests were made of the activity of expressed juice from mosaic-diseased plants receiving different amounts of nitrogen. The results of these tests are reported in this paper.

## Materials and methods

Turkish tobacco (*Nicotiana tabacum* L.) was used as the experimental host plant. Uniform seedlings in the 3-leaf stage were selected and transplanted to washed quartz sand or composted soil in 4-inch porous clay pots. The temperature of the greenhouse in which the plants were grown was held between 70° and 80° F. during the day and between 70° and 75° F. at night. The sand-grown plants were supplied with nutrient solutions containing either 1, 20, or 200 mg. of nitrogen per 100 ml. of solution, according to methods previously described (17). The medium-nitrogen level produced vigorous plants comparable to those grown in composted soil. The low and high levels were used in order to test the effect of a restricted supply of nitrogen and the effect of an amount in excess of that required for good growth. The high level retarded normal plant development but produced no other evidence of toxicity.

Nutrient application was started on the third day after transplanting. Between applications the plants were watered whenever necessary. The composition of the 3 nutrient solutions is shown in table I. In addition to the salts listed, boron and manganese were added to all solutions at a concentration of 0.5 p.p.m. of each as  $H_3BO_3$  and  $MnSO_4 \cdot 2H_2O$ , respectively. Representative plants in each group were inoculated with ordinary tobacco-mosaic virus, on the third day after transplanting, by rubbing the leaves

<sup>1</sup> The expression virus concentration or content as used in this paper refers only to the virus content in the expressed juice. While changes in the virus content in the juice probably parallel similar changes in the entire plant, this relationship has yet to be shown.

TABLE I  
COMPOSITION OF NUTRIENT SOLUTIONS

STOCK SOLUTIONS (0.5 MOLAR)	VOLUME OF STOCK SOLUTIONS PER LITER OF NUTRIENT SOLUTION		
	LOW-NITROGEN SOLUTION	MEDIUM-NITROGEN SOLUTION	HIGH-NITROGEN SOLUTION
	<i>ml.</i>	<i>ml.</i>	<i>ml.</i>
KH <sub>2</sub> PO <sub>4</sub> .....	12.9	12.9	.....
Ca(NO <sub>3</sub> ) <sub>2</sub> .....	0.5	11.0	63.0
MgSO <sub>4</sub> .....	4.0	4.0	4.0
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> .....	0.2	3.3	.....
CaCl <sub>2</sub> .....	11.0	.....	.....
NH <sub>4</sub> H <sub>2</sub> PO <sub>4</sub> .....	.....	.....	12.9
NH <sub>4</sub> NO <sub>3</sub> .....	.....	.....	67.0
KNO <sub>3</sub> .....	.....	.....	12.9

with a cheese cloth pad moistened with infectious juice. Typical symptoms of mosaic appeared about 5 days later on all plants inoculated. The experiment was carried out during October and November. The results reported are comparable with those obtained in earlier preliminary experiments.

### Experimentation

#### GROWTH OF PLANTS

Ten representative healthy and diseased plants from each treatment were harvested at 15, 25, and 35 days following inoculation. The heights of the plants and green weights of leaves were recorded at time of harvest. Dry weights were determined after the leaves had been heated for 10 hours at 100° C. and then dried to constant weight at 80° C. The relative growth of the plants at the first, second, and third harvests is illustrated in figure 1. The 4 plants on the left in each row were healthy, the 4 on the right were diseased. In each group of 4 plants, the first, from left to right, had received the low-nitrogen solution, the second the medium-nitrogen solution, and the third the high-nitrogen solution. The fourth plant in each group was the control grown in composted soil.

Table II gives the growth data obtained on the diseased plants at each of the 3 harvests. When harvested the diseased plants were smaller and weighed from 40 to 60 per cent. less than healthy plants on similar nutrient treatments. After 25 days, the diseased plants in sand on the medium-nitrogen treatment were practically the same size as the control plants in soil. After 35 days, however, the plants in sand were much taller and weighed about 30 per cent. more than the soil-grown plants. At all harvests the plants on the high-nitrogen treatment were smaller than those grown in soil. The low-nitrogen plants remained practically dormant over the entire

period of 35 days. It is of interest to note the percentage dry weight of the leaves on the several treatments, as recorded in table II. The high-nitrogen plants had the highest percentage dry weight and the control plants in soil had the lowest. This difference in moisture content is probably due to the

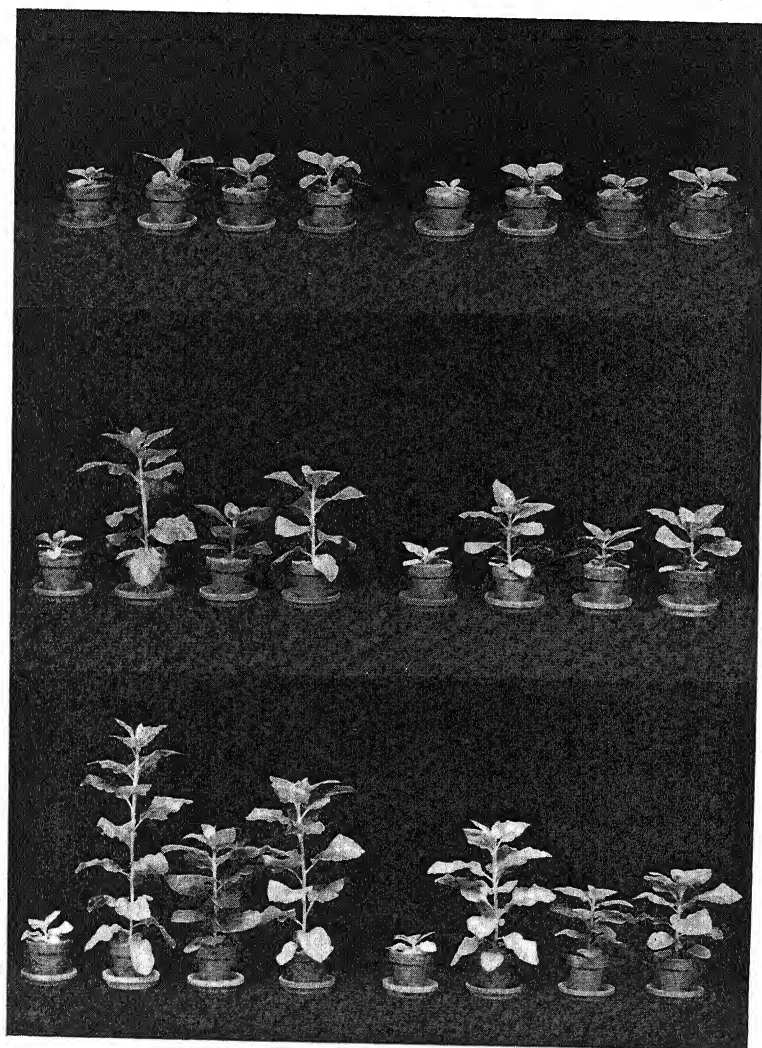


FIG. 1. Typical tobacco plants at time of harvest after 15, 25, and 35 days of treatment, respectively, from top to bottom. The 4 plants on the left in each row were healthy, the other 4 diseased with tobacco mosaic. In each group, the 4 treatments were low nitrogen, medium nitrogen, high nitrogen, and control in soil, respectively, from left to right. (Photograph by J. A. CARLILE.)

TABLE II

EFFECT OF NITROGEN ON GROWTH OF DISEASED TOBACCO PLANTS HARVESTED AT  
3 INTERVALS FOLLOWING INOCULATION

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULA- TION	GROWTH RECORDS AT TIME OF HARVEST			PERCENT- AGE DRY WEIGHT OF LEAVES
		HEIGHT	GREEN WEIGHT OF LEAVES	DRY WEIGHT OF LEAVES	
	<i>days</i>	<i>cm.</i>	<i>gm.</i>	<i>gm.</i>	<i>%</i>
Low .....	15	1*	1.8*	0.11*	6.1
Medium .....	15	2	6.0	0.39	6.5
High .....	15	2	4.9	0.40	8.2
Control .....	15	4	7.0	0.43	6.1
Low .....	25	1	3.2	0.24	7.5
Medium .....	25	12	19.8	1.48	7.5
High .....	25	4	8.0	0.72	9.0
Control .....	25	12	19.5	1.17	6.0
Low .....	35	2	4.1	0.30	7.3
Medium .....	35	31	36.4	2.67	7.3
High .....	35	12	18.3	1.64	9.0
Control .....	35	19	28.3	1.85	6.5

\* Average of 10 plants in each group.

high osmotic pressure exerted by the salts in the high-nitrogen solution, making it difficult for the plant to take up water from the solution. The osmotic pressure of the soil solution was probably quite low.

#### VIRUS ACTIVITY OF JUICE

Diseased plants similar to those used for growth measurements were cut at each harvest and the leaves frozen in a cold room held at  $-14^{\circ}$  C. On the following day they were thawed and minced in a food chopper. The juice was expressed from the pulp through cheese cloth, under pressure from a small hand press. The extracted juice was placed in sealed tubes and kept frozen at  $-14^{\circ}$  C. until used. Before being tested for virus activity, an aliquot of the juice was thawed and cleared of all insoluble materials by low-speed centrifugation.

The virus activity of the cleared juice was determined by the local-lesion method (8) modified, as suggested by SAMUEL and BALD (14), so that 2 samples were tested on opposite halves of the same leaf, thereby eliminating much of the variation attributable to differences in the susceptibility of individual plants. *Phaseolus vulgaris* L. var. Early Golden Cluster was used as a test plant, since PRICE (12) has shown that plants of this variety are suitable for measuring concentration of tobacco-mosaic virus. Nine- or 10-day-old bean plants were selected for uniformity in the size and development of primary leaves. These leaves were inoculated by rubbing the upper surface of each with a sterile pad of cheese cloth moistened with the solution

to be tested. Only 4 samples were compared at any one time, making it possible to test each sample once on every plant. Dilutions of the cleared juice were made with 0.1 M potassium phosphate buffer at pH 7.0. The lesions were counted 5 days after inoculation.

The total numbers of lesions produced on 36 half-leaves by each virus sample at dilutions of 1/100, 1/300, and 1/1000 are shown in table III.

TABLE III

VIRUS CONCENTRATION OF JUICE EXTRACTED FROM DISEASED TOBACCO PLANTS RECEIVING DIFFERENT AMOUNTS OF NITROGEN

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULATION	VOLUME OF JUICE EXTRACTED PER PLANT	NUMBER OF LESIONS ON 36 HALF LEAVES AT A DILUTION OF			RELATIVE VIRUS CONTENT* PER	
			1/100	1/300	1/1000	ML. OF JUICE	PLANT
	<i>days</i>	<i>ml.</i>					
Low .....	15	1.4	698	555	438	0.4	0.12
Medium .....	15	4.8	1252	764	536	1.4	1.5
High .....	15	2.8	1186	807	560	1.5	0.9
Control .....	15	4.5	1064	702	507	1.0	1.0
Low .....	25	1.8	350	306	177	0.04	0.005
Medium .....	25	16.8	946	830	566	2.0	2.3
High .....	25	4.8	1044	914	674	3.1	1.0
Control .....	25	14.3	894	720	405	1.0	1.0
Low .....	35	2.6	66	113	59	0.03	0.004
Medium .....	35	27.5	900	598	186	1.4	2.0
High .....	35	11.7	945	818	291	2.5	1.5
Control .....	35	19.4	717	496	177	1.0	1.0

\* Calculated from dilution curves as described in text.

There are several ways in which these lesion count data may be used to estimate the difference in concentration between virus samples tested at any one time. A comparison of the number of lesions produced at any one dilution shows which sample is most concentrated. The magnitude of the differences in concentration cannot be expressed by the differences in number of lesions, because, as is well known, the numbers of lesions produced are not directly proportional to the dilution of virus used for inoculation. When lesion count data at several dilutions are plotted on logarithmic paper, however, the points will lie along a straight line with a slope less than unity. The actual value of the slope will vary, depending upon the conditions under which the data are obtained. Consequently, it is necessary to take this fact into consideration in obtaining a mathematical comparison of the relative virus content of the samples. The method of calculation finally adopted may be illustrated with the data recorded at the first harvest 15 days after inoculation.



A straight line of the form

$$\text{Log } Y = b + a \log X$$

where  $Y$  is the number of lesions,  $X$  is the dilution, and  $a$  and  $b$  are constants, was fitted to the data from all 4 virus samples at each of the 3 dilutions. The equation obtained was:

$$\text{Log } Y = 3.612 + 0.304 \log X$$

The slope of this line, 0.304, may be regarded as the mean for all 4 virus samples. In a similar manner, straight lines were fitted to the data for each of the 4 samples. The values obtained were:

Low-nitrogen treatment .....	$\text{Log } Y = 3.247 + 0.202 \log X$
Medium-nitrogen treatment .....	$\text{Log } Y = 3.818 + 0.367 \log X$
High-nitrogen treatment .....	$\text{Log } Y = 3.722 + 0.326 \log X$
Control .....	$\text{Log } Y = 3.659 + 0.321 \log X$

The slopes of these 4 lines are similar, varying between 0.202 and 0.367. It is probable that this variation is mostly attributable to experimental error, inasmuch as the same virus was used throughout and inoculations were made at the same time. Using the mean slope, 0.304, and the mid-points of each of the 4 lines (*i.e.*, 2.742, 2.900, 2.907, and 2.856, respectively), the following equations were calculated:

Low-nitrogen treatment .....	$\text{Log } Y = 3.502 + 0.304 \log X$
Medium-nitrogen treatment .....	$\text{Log } Y = 3.660 + 0.304 \log X$
High-nitrogen treatment .....	$\text{Log } Y = 3.667 + 0.304 \log X$
Control .....	$\text{Log } Y = 3.616 + 0.304 \log X$

From these equations it is possible to calculate for any value of  $Y$  the corresponding value of  $X$ . For example, the degree of dilution ( $X$ ) necessary to produce a given number of lesions ( $Y$ ), such as 700, would be for the low-nitrogen treatment, 1/145; medium-nitrogen treatment, 1/480; high-nitrogen treatment, 1/506; and control, 1/344. The virus content of each virus sample when expressed according to the relative dilutions needed to produce a given number of lesions, was thus found to be 0.4, 1.4, 1.5, and 1.0, respectively.

In a consideration of table III, it is possible to compare with one another only those treatments that occur within any one of the 3 groups, because in the virus activity studies the concentration of only 4 treatments was tested at any one time. At 15 days after inoculation, the virus concentration (content per ml. of juice) of high-nitrogen plants did not differ significantly from that of plants on the medium-nitrogen treatment. With older plants, however, the virus concentration produced on the high-nitrogen treatment was appreciably higher than that produced on the medium-nitrogen treatment. Moreover, the plants in sand receiving nitrogen yielded juice with a much higher virus concentration than that from control plants grown in

soil. Another point of interest is the low virus concentration in mature plants deficient in nitrogen. The amount of virus in this juice was only about 1/30 that in juice from plants grown in soil and about 1/80 that of plants receiving the high-nitrogen solution. In general, the data show that the amount of virus extractable per ml. of juice is definitely correlated with the amount of nitrogen supplied to the plant. That is to say, plants deficient in nitrogen had a low concentration of virus, while plants receiving an ample supply had a high concentration.

The effect which these nitrogen levels have on the total amount of virus in the expressed juice from each plant is shown in the last column of table III. The virus content per plant was calculated by multiplying the relative virus concentration by the total number of ml. of juice extracted from each plant. These data show that the nitrogen treatments in sand produced plants with a virus content higher than that produced in soil-grown plants. The virus content was not dependent primarily upon the actual growth of the plants. That is to say, the plants in soil were nearly twice the size of those in sand on the high-nitrogen treatment, yet they had only about  $\frac{2}{3}$  as much total virus in their expressed juice.

The data of table III do not allow a direct comparison between virus concentration of plants on the same treatment but of different ages, since virus activity of these groups was determined at different times. For the purpose of making such a comparison, additional tests were carried out. The experimental data are presented in table IV. In each group, the virus

TABLE IV

CONCENTRATION OF VIRUS IN JUICE EXTRACTED FROM DISEASED TOBACCO PLANTS  
HARVESTED 15, 25, AND 35 DAYS FOLLOWING INOCULATION

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULA- TION	NUMBER OF LESIONS ON 36 HALF LEAVES AT A DILU- TION OF			RELATIVE VIRUS CONTENT PER	
		1/100	1/300	1/1000	ML. OF JUICE	PLANT
	<i>days</i>					
Low .....	15	877	472	325	1.0	1.0
Low .....	25	535	187	67	0.2	0.3
Low .....	35	343	124	85	0.15	0.3
Medium .....	15	1745	1145	543	1.0	1.0
Medium .....	25	1343	893	513	0.6	2.1
Medium .....	35	1193	869	570	0.6	3.4
High .....	15	1275	778	574	1.0	1.0
High .....	25	1339	968	770	2.0	3.4
High .....	35	1187	1014	654	1.5	6.3
Control .....	15	1683	769	654	1.0	1.0
Control .....	25	1607	1111	658	1.3	4.1
Control .....	35	1285	877	608	0.8	3.4

concentration of the juice at the first harvest is taken as unity. Only 3 samples were tested at any one time. On the low- and medium-nitrogen treatments, the virus concentration was higher 15 days after inoculation than it was after 25 or 35 days. In the high-nitrogen plants, the virus concentration after 25 days was double that of the first harvest 10 days earlier. Ten days later, however, the virus concentration had decreased about 25 per cent. In the control plants in soil, the virus concentration reached a maximum in about 25 days and then decreased about 50 per cent. during the next 10 days.

The effect which these nitrogen treatments have on the total amount of virus in extracted juice from leaves of each plant is shown at the right in table IV. In low-nitrogen plants, the virus content of the expressed juice decreased about  $\frac{2}{3}$  during the 20 days following the first harvest, even though the plants more than doubled in size during this period (table II). In the control plants and those on the medium-nitrogen treatment, the virus content increased about 3 times, whereas growth increased 4 or 5 times in the 20-day interval between the first and third harvests. In the high-nitrogen plants, the virus increased over 6-fold, whereas growth increase was only 4-fold during this 20-day period.

#### NITROGEN CONTENT OF JUICE

In order to determine whether there was any relationship between the virus concentration and nitrogen content of the extracted juice, aliquots of the juice were analyzed for total nitrogen and protein nitrogen. The aliquots used were from the same lots of juice used in the virus activity studies described above. The juice was thawed and then cleared of all insoluble materials in the same way by low-speed centrifugation. Analyses were also carried out on aliquots from juices of healthy plants.

Total nitrogen was determined by a modification of the method outlined by RANKER (13). The method, adapted to micro-determinations, was as follows: Two ml. of plant juice were placed in a 125-ml. Kjeldahl flask and evaporated to dryness in an oven held at 80° C. Salicylic acid (0.15 gm.) and concentrated  $\text{H}_2\text{SO}_4$  (4 ml.) were added and the mixture allowed to stand overnight with occasional shaking. Zinc dust (0.15 gm.) and a few alundum chips were then added and the mixture boiled gently over a micro-burner for about 10 minutes. Kjeldahl nitrogen was then determined by the method of FOLIN and FARMER (7) as modified by NORTHROP (10) and LAURO (9). To the reduced solution was added 1 gm. of  $\text{K}_2\text{SO}_4$  and 2 drops of  $\text{SeOCl}$  and the mixture digested for at least 5 minutes after the contents had become clear or straw-colored. Thirty ml. of water were added and the solution allowed to cool. After the addition of 12 ml. of a 50 per cent. NaOH solution, nitrogen as ammonia was distilled over into 0.02 N standard

acid. With methyl red as an indicator, all titrations were made with 0.02 N NaOH to the complete disappearance of any red tinge.

Protein nitrogen was determined as follows: 4 ml. of juice were treated with 4 ml. of hot 5 per cent. trichloroacetic acid. The suspension was immediately cooled and then centrifuged. The denatured protein was dissolved in 1 ml. of 0.2 N NaOH and reprecipitated with 1 ml. of cold 10 per cent. trichloroacetic acid. Following centrifugation, the pellet was dissolved in 1 ml. of 0.2 N NaOH and transferred to a 125-ml. Kjeldahl flask. Kjeldahl nitrogen was then determined, as already described, by adding concentrated  $H_2SO_4$ , alundum chips,  $K_2SO_4$ , etc.

From the data in table V, it is obvious that there is a decided variation, not only in the total-nitrogen but also in the protein-nitrogen content of the

TABLE V

NITROGEN ANALYSES OF JUICE FROM FROZEN HEALTHY AND DISEASED TOBACCO PLANTS HARVESTED 15, 25, AND 35 DAYS AFTER INOCULATION

NITROGEN LEVEL	TIME OF HARVEST: DAYS AFTER INOCULATION	AVERAGE GREEN WEIGHT OF LEAVES		NITROGEN PER ML. OF JUICE			
				TOTAL NITROGEN		PROTEIN NITROGEN	
		HEALTHY	DISEASED	HEALTHY	DISEASED	HEALTHY	DISEASED
	days	gm.	gm.	mg.	mg.	mg.	mg.
Low .....	15	3.3	1.8	0.20	0.35	0.01	0.21
Medium .....	15	12.8	6.6	1.26	1.46	0.22	0.60
High .....	15	7.4	3.9	2.34	2.33	0.14	0.46
Control .....	15	12.1	6.0	1.04	1.13	0.15	0.42
Low .....	25	4.9	2.3	0.18	0.28	0.003	0.10
Medium .....	25	28.6	21.6	0.61	1.15	0.15	0.52
High .....	25	17.8	6.7	2.64	3.36	0.13	0.73
Control .....	25	37.2	17.7	0.68	0.89	0.09	0.40
Low .....	35	6.7	3.7	0.20	0.25	0.005	0.04
Medium .....	35	49.2	37.7	0.63	1.06	0.15	0.57
High .....	35	35.7	17.7	3.08	3.80	0.14	0.77
Control .....	35	62.5	26.0	0.46	0.65	0.10	0.39

juice from diseased plants, depending on the nutrient treatment. The total-nitrogen content (per ml. of juice) was correlated with the amount of nitrogen supplied to the plants. The nitrogen content of all plants except those on the high-nitrogen treatment was higher at the first harvest 15 days after inoculation than at either of the 2 subsequent test periods. With the high-nitrogen plants, however, the nitrogen content was higher 35 days after inoculation than it had been after 25 or 15 days. The plants on the low-nitrogen treatment had very little nitrogen in their extracted juice.

The protein-nitrogen fraction showed the same general correlation as exhibited by the total-nitrogen content. The only exception was the low

protein-nitrogen content of the high-nitrogen plants at the first harvest. It is of interest to note that, with the exception of the plants on the high-nitrogen treatment, the protein nitrogen accounted for about 50 per cent. of the total nitrogen present in the juice. In the high-nitrogen plants, however, the protein nitrogen accounted for only 20 per cent. of the total nitrogen, indicating that there was present in these plants a much larger reserve of inorganic nitrogen available for assimilation.

Although the nitrogen content in juice from healthy plants was lower than that in juice from diseased plants on corresponding treatments, the variations among treatments were of the same general order. In healthy juice, however, the protein nitrogen accounted for only a small part of the total nitrogen present in the juice. In the low-nitrogen plants the protein nitrogen accounted for about 5 per cent. of the total nitrogen, in medium-nitrogen plants about 18 per cent., in high-nitrogen plants about 5 per cent., and in the control plants about 18 per cent. Moreover, the total-nitrogen content of juice from the high-nitrogen plants was higher than that from the medium-nitrogen plants, but the protein-nitrogen content was slightly lower.

The data in tables III and V show that there is a general correlation between virus concentration and nitrogen content in juice from diseased plants. In any one harvest, the juice with the highest virus concentration was always characterized by having the largest amount of both total and protein nitrogen. On the other hand, the juice with the lowest virus concentration had the smallest amount of nitrogen. These analyses offer definite evidence that the low virus activity in juice from low-nitrogen plants is due to a limitation in the nitrogen supply.

#### Discussion

It has commonly been supposed that the altered disease complex, brought about by variations in the nitrogen nutrition of the host, is due primarily to a change in the susceptibility of the host. Little attention has been given to the possibility that the causal agent of the disease may also be affected in some way by the mineral nutrition of the host. A review of the literature reveals several papers (4, 15, 19, 20) dealing with the effect of nitrogen on the severity of virus diseases, but no paper dealing with the effect of nitrogen on the concentration of the causative agent. Although such criteria as intensity of disease symptoms and retardation of growth show that host metabolism is altered during the course of the disease, they offer no evidence that the concentration of the causative agent is affected in any way.

The experimental data herein reported show that there are very definite variations in the concentration of the virus in juices of plants receiving different amounts of nitrogen. With our present knowledge, it is impossible

to say whether these variations are brought about directly by differences in the amounts of nitrogen supplied to the host or by changes in the physiological or morphological structure of the plant caused by these differences in nitrogen nutrition. It is obvious, however, that the ultimate virus concentration has been greatly affected in some way by the nitrogen supply. In view of these findings, the apparent increase in host susceptibility brought about by added nitrogen may be attributed, at least in part, to the increased content of virus present in these plants.

Competition between the normal growth processes and those responsible for virus increase may possibly be a factor in governing the extent to which virus is formed. When the supply is limited, most of the available nitrogen may be utilized for plant growth. As more and more nitrogen is made available, the supply of nitrogen above that needed for growth processes becomes greater, and this reservoir of nitrogen may account for the high virus content in plants on the high-nitrogen treatment. The nitrogen analyses show that plants on the high-nitrogen treatment contained a large amount of non-protein nitrogen that was presumably available for either plant growth or virus increase. With this large reservoir of nitrogen, it might be expected that the virus content in these plants would be higher than that demonstrated experimentally. Since protein content and virus activity are apparently closely associated, it is not improbable that the presence of some other element, such as phosphorus, in insufficient quantities may be a factor in limiting protein formation and also in limiting a further increase in the virus content of the high-nitrogen plants.

The experimental data show that the time required for the virus concentration to increase to a maximum in plant juice varied with the nitrogen supplied to the plant. This maximum was reached earlier with a low or medium supply of nitrogen than with an excess of nitrogen. Following this increase to a maximum, there appeared to be a decrease in virus content. The evidence indicates, therefore, that the supply of available nitrogen may control not only the increase but also the subsequent decrease in virus content. With a moderate supply of nitrogen, virus may increase more slowly than growth, resulting in an apparent decrease in virus concentration. With an excess of nitrogen, virus increase apparently continues unhampered but growth is retarded to some extent.

The experimental data show that juice from diseased plants had a much higher nitrogen content than that from corresponding healthy plants. These data are in agreement with those previously reported by other workers (1, 2, 3, 5, 6, 11, 18). The significance of this finding is difficult to evaluate with any degree of certainty. It is necessary to bear in mind that the nitrogen analyses recorded in table V are a measure of the soluble nitrogen in the juice after freezing, and not a measure of the total nitrogen either in the



plant as a whole or in freshly extracted juice. It is worthy of note, in this connection, that the difference between the total-nitrogen content in juices of healthy and diseased plants approximates very closely the difference in protein-nitrogen contents of the same juices. It is not improbable that some proteinaceous materials present in the juice of healthy plants may be relatively unstable and may become denatured by freezing and thereby rendered insoluble. This protein fraction would then be removed from the juice by the subsequent centrifugation used to clear the juice prior to analysis. Corroboration of this interpretation may be found in recent work of BAWDEN and PIRIE (3) who showed that a high molecular weight protein present in freshly extracted juice of healthy plants was converted irreversibly into an insoluble material when the juice was subjected to freezing.

### Summary

The influence of nitrogen nutrition on virus production in Turkish tobacco plants was studied by supplying them with nutrient solutions containing either a low, medium, or high level of nitrogen, inoculating them with tobacco-mosaic virus, and measuring the virus activity in juice from frozen plants harvested at 3 different intervals following inoculation.

The virus activity of expressed juice was directly correlated with the amount of nitrogen supplied to the plants. The virus concentration of juice from plants receiving an ample nitrogen supply was over 80 times that of juice from nitrogen-deficient plants. Moreover, the virus content of expressed juice from plants in sand receiving the medium-nitrogen solution was more than double that found in juice from plants grown in composted soil. It is not improbable that the low virus activity of expressed juice from nitrogen-deficient plants is brought about by the limitation in nitrogen supply.

With insufficient nitrogen, the total virus content in extracted juice decreased following the initial systemic spread of the virus through the plant. With an over-abundance of nitrogen, the virus content increased at a faster rate than that at which the plants grew.

The increase in virus content showed no apparent correlation with the growth of the plants. Although the soil-grown plants were much larger than those in sand that received the high-nitrogen solution, they had only about  $\frac{2}{3}$  as much total virus in their expressed juice.

The total-nitrogen and protein-nitrogen contents in the expressed juice varied directly with the nitrogen level of the nutrient solution. These amounts were much larger in juice from diseased plants than in juice from healthy plants on corresponding treatments. Virus activity was directly correlated with the nitrogen content in juice of diseased plants. The juice

with the highest total- and protein-nitrogen contents was found to have the highest virus activity.

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# FLORAL DEVELOPMENT OF CERTAIN SPECIES AS INFLUENCED BY X-RADIATION OF BUDS

EDNA LOUISE JOHNSON

(WITH TWO FIGURES)

## Introduction

X-radiation of susceptible plants which are nearing the reproductive stage usually causes abscission of buds or certain types of anomalies in flower structure. An earlier paper by the writer (3) reports the occurrence of abnormalities of floral parts including the production of double flowers when tomato plants were given three X-ray doses previous to blossoming. A single heavy dose usually causes bud abscission; later growth may produce blossoms some of which are normal, while others are double or triple.

GOODSPEED (1) noted that in *Nicotiana langsdorffi* buds of the terminal inflorescence were abscised immediately after treatment and all the first flowers on laterals were abnormal. After a short time, only normal flowers were produced. MOORE and HASKINS (4) reported that irradiated petunia buds gave rise to blossoms displaying various anomalies.

The present article discusses various types of abnormalities in flower structure which result when young buds are irradiated with one medium dose. A rather extended analysis of the relationship found to exist between the degree of bud development at the time of treatment and the occurrence and character of the anomalies produced will be given for one of the species used in the experimentation.

## Materials and methods

Plants of *Phlox drummondii*, *Nicotiana tabacum*, and *Salpiglossis sinuata* on which the young buds had developed were irradiated with one medium dose of X-rays; records were then taken of the dates of blooming together with a statement concerning the occurrence of either normal flowers or of those with floral abnormalities. If the latter were present, a brief description of each was given. The exact size of the buds of the first two species was not recorded at the time of irradiation. It seemed desirable, however, to use the third species, *Salpiglossis*, to determine whether there is a definite relationship between the degree of bud development and the type of abnormality produced. For this study, over 1500 blossoms from irradiated and control buds were used. The lengths of all buds one centimeter or over, and the number of all those under one centimeter, were recorded; these buds were then tagged and numbered as were also those developing later on the vegetative tissue which was not present at the time of treatment. Accurate records could thus be kept of the time of opening of each bud and of the character of the floral anomaly, if any appeared.

## Experimentation

## PHLOX

Three groups of *Phlox drummondii* plants were given doses of 2000, 1800, and 1500 r-units respectively. Well-developed buds on plants receiving 2000 r-units survived and usually developed normally; the small buds which were present, abscised. About the sixth week after treatment, blossoms developed from the new growth formed after irradiation of the plants. Floral anomalies frequently seen from the sixth to the eighth week included: (1) increase in number of corolla lobes; (2) occurrence of white streaks or spots on corolla lobes; and (3) a peculiar appearance of the corolla which has been referred to as "stippled" because the corolla looked as if it had been marked with tiny white dots. By the ninth week normal blossoms appeared in abundance, although some anomalies were evident up to the twelfth week when the experiment was concluded.

In the group receiving 1800 r-units, the corolla lobes of some flowers in every pot were either blotched or stippled two weeks after treatment. During the following four weeks, most of the plants produced no more flowers; between the seventh and eighth week, the maximum number of anomalies was evident. Throughout the entire experiment, the presence of dissected margins or fimbriated corolla lobes was the most frequently occurring anomaly, constituting one-third of the total number. A reproduced painting of one of the blossoms which developed on a plant 22 days after irradiation with 1800 r-units (plate IV, C-2) shows this character. Comparison with a control (plate IV, C-1) shows not only difference in the lobe margins but also reduction in size. Increased number of lobes and "stippled" corolla were also of common occurrence.

The dose of 1500 r-units given to the plants of group III caused abscission of only a few buds; and many of the blossoms developing during the first 3.5 weeks had anomalous characters. Flower production was decreased after this period, however; but later when blooming was more abundant, irregularities again appeared; corolla lobes with fimbriated edges and those with white streaks and spots were most prevalent.

Considering all groups as a whole, the anomaly occurring with the greatest frequency was that of the fimbriated or dissected corolla lobe. Others in order of occurrence were: increased number of lobes, appearance of white streaks or spots on the corolla lobes, color changes, decreased number of lobes, and dwarf blossoms (table I).

## NICOTIANA

A four-months record of floral development from budded *Nicotiana tabacum* plants which were exposed to one X-ray dose of 2000 r-units is given in table II. Some type of abnormality was evident in 57.5 per cent.

TABLE I

FLORAL ANOMALIES OF PHLOX PLANTS IRRADIATED AFTER BUD DEVELOPMENT

ANOMALIES	GROUP I 2000 R-UNITS	GROUP II 1800 R-UNITS	GROUP III 1500 R-UNITS	TOTAL OF ALL GROUPS
	%	%	%	%
Dissected or fimbriated corolla lobes .....	15.1	33.7	53.7	30.0
Increased number of lobes .....	35.8	30.0	9.2	27.9
White streaks or spots .....	19.8	6.2	18.5	15.0
White "stippling" on lobes .....	17.9	15.3	7.4	14.6
Color change in lobes .....	6.6	10.0	11.1	8.7
Decreased number of lobes .....	.9	5.0	0.0	2.1
Dwarf blossoms .....	3.8	0.0	0.0	1.7

of the blossoms from irradiated plants; the greatest number of these occurred during the first 20 days after irradiation and again in the period extending from 41 to 60 days. In general it was found that buds which were large enough to show color at the time of irradiation developed normally; those which were very small would either abscise or, after unfolding, show some anomaly. After the first 20 days, flowering was decreased until blossoms formed from buds which developed on new tissue appearing after treatment. Such blossoms which opened about the 40th day, showed the maximum number of irregularities; hence the largest number of anomalies is listed for the 41- to 60-day period. Normal blossoms began to appear in greater numbers in the 61- to 80-day period which was toward the end of the blossoming cycle, the average length of which was 65.7 days. The total flowering period for the different plants varied greatly, for in some cases scarcely any flowers developed after irradiation; in two-thirds of the irradiated plants, however, after abscission of buds borne on the original main stem, new branches formed which continued to bear blossoms.

About 30 per cent. of the anomalies are listed as "color change," the term being used to designate either a uniform lighter color which was characteristic of the entire flower or to indicate a light sector in one of the lobes (plate IV, A-4, 5). These color changes were evident throughout the entire experimental period, occurring with the greatest frequency at the time when the greatest number of anomalies was evident. Many of the blossoms which opened during the first 20 days showed the stippled appearance which was more pronounced than that shown in the Phlox (plate IV, A-2). In some cases these tiny white dots developed into streaks; in a considerable number of instances, however, prominent white streaks or spots were evident when the blossoms first unfolded (plate IV, A-3, 4). Stippling, spotting, and streaking were most common from the 21st to 60th day. GOODSPEED (2) has

TABLE II  
FLORAL DEVELOPMENT OF NICOTIANA PLANTS

	PLANTS EXPOSED TO 2000 R-UNITS						
	1-20 DAYS	21-40 DAYS	41-60 DAYS	61-80 DAYS	81-120 DAYS	TOTAL NO.	PERCENT- AGE
A. ANOMALIES OCCURRING IN BLOSSOMS FROM PLANTS IRRADIATED AFTER DEVELOPMENT OF BUDS							
Color change .....	3	1	8	4	7	23	29.48
Streaked .....	1	5	5	1	0	12	15.38
Stippled .....	9	2	0	0	0	11	14.10
Lobes unlike in size .....	6	1	2	0	0	9	11.53
Spotted .....	3	4	0	0	0	7	8.97
Tube split .....	1	0	4	1	0	6	7.7
Dwarf .....	1	2	1	1	0	5	6.41
Lobes increased in number .....	0	0	4	0	0	4	5.12
Lobes decreased in number .....	0	0	1	0	0	1	1.28
Total number .....	24	15	25	7	7	78	
Percentage of to- tal .....	30.8	19.2	32.0	9.0	9.0		
B. NORMAL BLOSSOMS FROM PLANTS IRRADIATED AFTER DEVELOPMENT OF BUDS							
Total number .....	6	3	13	20	9	51	
Percentage of to- tal .....	11.8	5.9	25.5	39.2	17.6		
CONTROLS (BLOSSOMS ALL NORMAL)							
Total number .....	21	22	20	7	4	74	
Percentage .....	28.4	29.7	27.0	9.4	5.4		

figured tobacco flowers with white dots and lines on the corolla lobes and speaks of them as representing areas of air-filled cells.

Variations in number and size of corolla lobes were numerous. Permanent decreased size of upper lobes was marked in some specimens. Increase in corolla-lobe number (plate IV, A-6) over that of the normal was responsible for five per cent. of the anomalies while but one per cent. showed a reduced number of lobes. Cases of split corolla tube, often accompanied by abnormal curvature and marked dwarfness, were other unusual features in the blossoms of irradiated plants.

The production of blossoms by controls was rather evenly distributed over the first 60 days. All of the flowers were normal except two which showed a few white spots.



## SALPIGLOSSIS

*Salpiglossis sinuata*, the third species which was treated in the budded stage, was also given 2000 r-units. At the time of irradiation, the lengths of all buds one centimeter or over in length and the number of those under one centimeter were recorded. A numbered tag was attached to each bud; when the blossom opened, records were made of the date and appearance of each flower.

Over 1500 blossoms from irradiated buds and controls were thus observed over a period of three months. Results are given for 991 blossoms on the irradiated plants and 453 on the controls. Cases were not included where records were incomplete as to the size of buds at time of irradiation. Two plants were frequently grown in one pot; when one had buds of the size desired for radiation, the second was often small with few buds visible. The plants which were more mature at the time of irradiation were placed in group I while those which were less well developed were considered as group II. Though the types of anomalies produced were practically the same in both cases, it seemed best to record the results separately, as plants in group II were often slightly less vigorous than those of the first group.

Of the 741 blossoms from the irradiated plants of group I, 25 per cent. were normal; the remainder exhibited 770 anomalies of ten different types (table III). The 20-day period in which the blossoms opened, and the bud size at the time of irradiation, have been considered in the tabulation. The anomalies are listed according to their frequency of occurrence and will be discussed in that order. A spotted condition of the corolla constituted 24.5 per cent. of all the abnormalities (plate IV, B-2, 5); in many cases, the spots which were very small and close together gave the appearance of stippling. Normally the corolla is of a velvety texture, but the occurrence of the dots

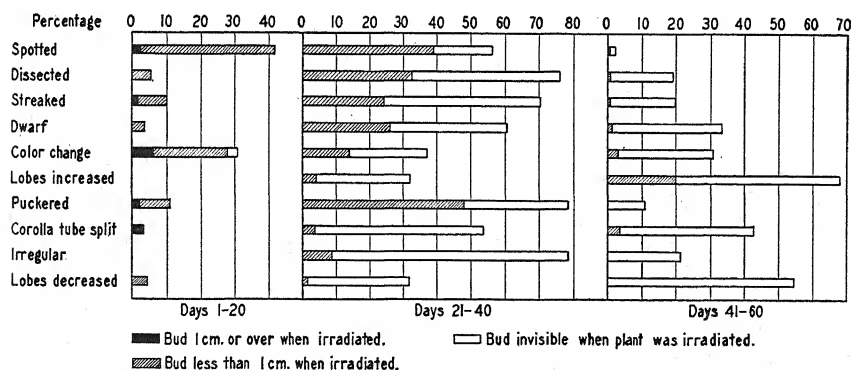


FIG. 1. Distribution of anomalies in flower structure produced by *Salpiglossis* plants exposed to X-radiation.

TABLE III  
PERCENTAGE OF ANOMALIES FOUND AMONG 741 FLOWERS PRODUCED BY 46 SALPIGLOSSIS PLANTS WHICH WERE EXPOSED TO 2500 R-UNITS OF X-RAYS  
(GROUP I)

TYPE OF FLORAL ANOMALY	DAYS 1-20 SIZE OF BUD WHEN IRRADIATED				DAYS 21-40 SIZE OF BUD WHEN IRRADIATED				DAYS 41-60 SIZE OF BUD WHEN IRRADIATED				DAYS 61-80 BUDS ALL INVISI- BLE TOTAL	TOTAL PER CENT. OF ALL ANOMALIES
	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	IN- VISI- BLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	IN- VISI- BLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	IN- VISI- BLE		
Spotted .....	% 41.8	% 6.3	% 93.7	% 0.0	% 56.1	% 0.0	% 69.8	% 30.2	% 2.1	% 0.0	% 25.0	% 75.0	% 0.0	% 24.5
Dissected .....	5.3	0.0	100.0	0.0	75.8	0.0	43.0	57.0	18.9	0.0	4.0	96.0	0.0	17.1
Streaked .....	9.9	15.4	84.6	0.0	70.2	0.0	34.8	65.2	19.8	0.0	3.8	96.2	0.0	17.0
Dwarf .....	3.6	0.0	100.0	0.0	60.7	0.0	43.1	56.9	33.3	0.0	3.8	96.2	2.4	10.9
Color change .....	30.8	20.0	70.0	10.0	36.9	0.0	37.5	62.5	30.8	0.0	10.0	90.0	1.5	8.4
Lobes increased .....	0.0				32.0	0.0	12.5	87.5	68.0	0.0	29.4	70.6	0.0	6.5
Puckered .....	10.9	20.0	80.0	0.0	78.3	0.0	61.1	38.9	10.9	0.0	0.0	100.0	0.0	6.0
Corolla tube split .....	3.6	100.0	0.0	0.0	53.6	0.0	6.7	93.3	42.9	0.0	8.3	91.7	0.0	3.6
Irregular .....	0.0				78.3	0.0	11.1	89.9	21.7	0.0	0.0	100.0	0.0	3.0
Lobes decreased .....	4.5	0.0	100.0	0.0	31.8	0.0	4.5	95.5	54.5	0.0	0.0	100.0	9.0	2.8

obscured this appearance and gave it a "frosty" look. A study of table III and figure 1 indicates that this spotted condition was very prevalent during the first 40 days and that it was a characteristic response in flowers from buds which were less than 1 cm. in length at the time of radiation. The dissected, ragged, or fringed appearance of the corolla lobes apparent in plate IV, B-5, 6 was especially common among flowers opening during the 21- to 40-day period. The greater number appearing in from 21 to 60 days were from buds which were invisible at the time of radiation (fig. 1).

Definite white or yellowish streaks on corolla tubes occurred most frequently in the 21- to 40-day period (fig. 1); the higher percentage in this period as well as in the later one was from buds which were invisible at the time of treatment. It may be noted that the percentage of streaked corollas occurring in the 1- to 20-day period was considerably less than the spotted ones. MOORE and HASKINS (4) found that *Petunia* buds irradiated about 16 days before the blossoms opened showed corollas with white stippling. In turn, there followed flowers with coarsely flecked corollas and others exhibiting white lines and wedges. Flowers developing later were normal. *Petunia*, however, was not reported as having many of the other morphological variations exhibited by flowers of the irradiated *Salpiglossis* plants.

Few dwarf blossoms occurred in the first 20-day period, the higher percentage being present in the 21- to 40-day period. The plants which continued their flowering for as long as 80 days, developed dwarf blossoms which came from buds which were invisible at the time of treatment.

The term "color change" is used to include conspicuous grayish margins apparent in some specimens, as well as changes in the color of certain lobes (plate IV, B-3). These color changes were about equally distributed between the first three 20-day periods. A few gray-margined blossoms were also evident among the control plants of this group.

In but two types of anomalies, increased and decreased number of corolla lobes, was the highest percentage of occurrence found in the 41- to 60-day period. The percentage of corollas with an abnormally large number of lobes was higher than for those with a decreased number; the latter irregularity was the most frequent one found during the 61- to 80-day period.

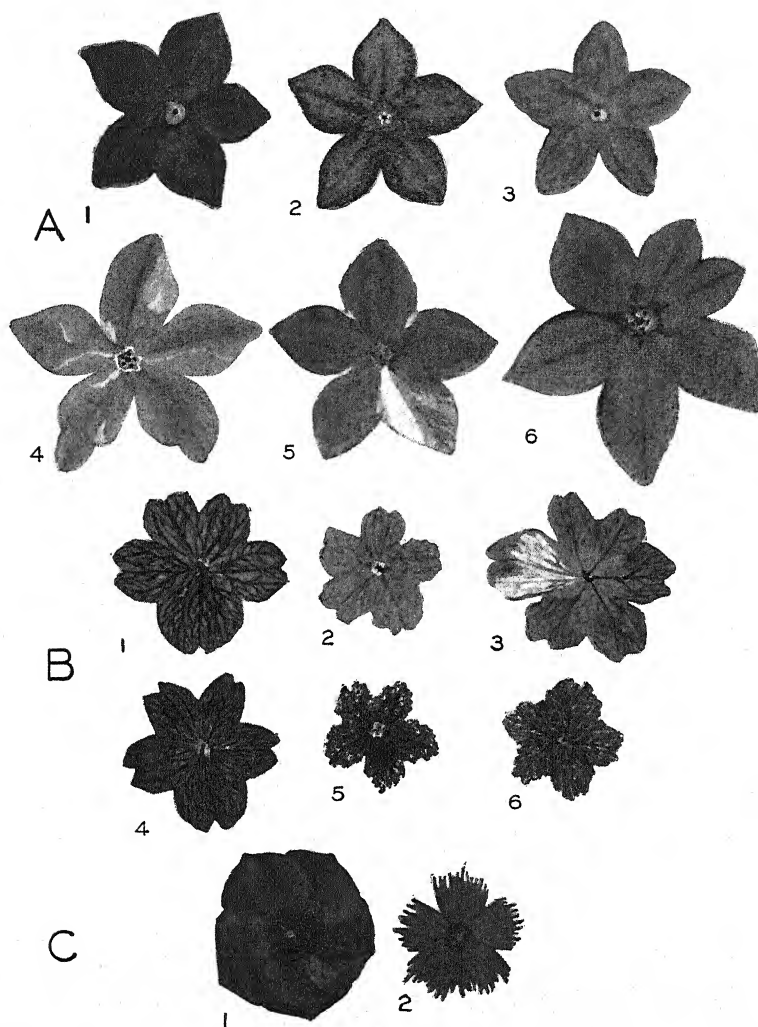
A "puckered" appearance of the corolla tissue similar to that seen in crepe cloth constituted 6 per cent. of all the anomalies of group I. This was a new type of anomaly not present in the *Phlox* or tobacco, but it has been reported by MORGAN (5) as occurring in blossoms developing from irradiated *Freesia* bulbs. The greater number of these puckered corollas occurred in the 21- to 40-day period, and the highest percentage came from buds less than 1 cm. in length at time of irradiation. Cases of split corolla tube occurred in *Salpiglossis* as well as in *Nicotiana*. They were more in evidence from the 21st to the 60th day; by far the greater number were from buds invisible at the time of irradiation (fig. 1).



## EXPLANATION OF PLATE IV

## BLOSSOMS OF PLANTS WHICH WERE X-RADIATED AFTER DEVELOPMENT OF BUDS

- A. Blossoms of *Nicotiana tabacum* from plants which were producing buds when X-radiated with a dose of 2000 r-units. 1, control; 2, blossom which opened 18 days after bud had received 2000 r-units; note stippled appearance of corolla lobes; 3, blossom which opened in 33 days from bud which was invisible at time of treatment; 4, blossom ( $\times 2.5$ ) which appeared on a secondary branch formed after treatment; light streaks and sectors are conspicuous; 5, blossom with absence of color in one lobe of corolla which opened 60 days after treatment; 6, blossom ( $\times 2.5$ ) with deeply divided lobe of corolla which opened 54 days after treatment; branch on which it grew had not formed at time of irradiation.
- B. *Salpiglossis sinuata* blossoms from plants which were producing buds when irradiated with a dose of 2000 r-units. 1, control; 2, dwarf blossom with spotted, puckered tissue which opened 35 days after treatment; 3, blossom with increase in lobe number as well as change in color; blossom opened 48 days after treatment; 4, control; 5, dwarf blossom with spotted, puckered tissue which opened 39 days after treatment; the bud at time of irradiation was invisible; 6, blossom which opened 26 days after treatment from bud under 1 cm. in length when irradiated.
- C. *Phlox drummondii*. 1, control ( $\times 2.5$ ); 2, blossom ( $\times 3$ ) with conspicuous fimbriated lobes which developed on plant 22 days after irradiation with 1800 r-units. Many cases of this anomaly occurred.



Johnson: X-radiation

TABLE IV  
TYPES OF ANOMALIES FOUND AMONG THE 250 FLOWERS PRODUCED BY 16 IRRADIATED SALPIGLOSSIS PLANTS WHICH WERE EXPOSED TO 2500 R-UNITS  
(GROUP II)

TYPE OF FLORAL ANOMALY	DAYS 1-20 SIZE OF BUD WHEN IRRADIATED				DAYS 21-40 SIZE OF BUD WHEN IRRADIATED				DAYS 41-60 BUDS ALL INVISIBLE TOTAL	TOTAL PER CENT. OF ALL ANOMALIES
	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVIS- IBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVIS- IBLE		
Spotted .....	% 51.6	% 10	% 90	% 0.0	% 46.9	% 0.0	% 40	% 60	% 1.6	% 25.6
Streaked .....	8.8	0.0	100	0.0	84.2	0.0	31.3	68.7	7.0	22.8
Color change .....	26.7	37.5	62.5	0.0	26.7	0.0	37.5	62.5	46.7	12.0
Dwarf .....	7.1	0.0	100	0.0	53.6	0.0	0.0	100.0	39.3	11.2
Dissected .....	3.6	0.0	100.0	0.0	89.3	0.0	8.0	92.0	7.1	11.2
Irregular .....	13.3	100.0	0.0	0.0	33.3	0.0	20.0	80.0	53.3	6.0
Lobes increased .....	0.0	0.0	0.0	0.0	72.7	0.0	0.0	100.0	27.3	4.4
Puckered .....	0.0	0.0	0.0	0.0	87.5	0.0	28.6	71.4	12.5	3.2
Lobes decreased .....	0.0	0.0	0.0	0.0	80.0	0.0	0.0	100.0	20.0	2.0
Corolla tube split .....	0.0	0.0	0.0	0.0	75	0.0	0.0	100.0	25.0	1.6

Cases listed as "irregular" refer to an irregularity in corolla lobes such as the appearance of a small lobe inside a large one, or a lobe abnormal in shape and size.

In the irradiated plants of group I and in the controls (table VI) the greater number of blossoms as well as the greater number of anomalies developed during the 21- to 40-day period; the smallest number in the 61- to 80-day period. The others were rather evenly distributed in the first and third periods.

Anomalous floral structures similar to those just discussed were also evident in the blossoms of group II. These have been classified and listed in table IV. It may be noted that there is but slight difference in the order of frequency of the occurrence of the anomalies for the two groups; thus results for the second group serve to confirm the conclusions drawn for the first. The summarized record of the two groups as shown in table V indicates that only 25 per cent. of the blossoms in the first group and 21 per cent. in the second group were normal. All others showed at least one type of abnormality. In both groups the greatest number, approximately 60 per cent. in each, were evident in the 21- to 40-day period. The highest percentage in both groups during this period was from the lot in which the buds at time of irradiation were invisible. The percentage of anomalies in the 1- to 20-day period and those in the 41- to 60-day period were practically the same in both groups. In the first period, blossoms from irradiated buds under 1 cm. in length had the highest percentage of anomalies; in the 41- to 60-day period, the group in which the buds were invisible at radiation contained the highest percentage.

One noticeable difference between the two groups is the fact that the irradiated plants of group I, which produced normal blossoms, bore the greater proportion of them during the first 20 days and from buds which were over 1 cm. in length at the time of treatment. Of the normals produced by irradiated plants of group II, the greater number were borne in the 21- to 40-day period and the highest percentage came from buds less than 1 cm. in length at time of treatment.

Blossoms from the 39 control plants numbered 454. Among the first group 10 per cent. showed anomalous characters, the chief of which was color change (table VI). Among plants of the second group, 13 per cent. gave evidence of some irregularities, chief of which were color changes and irregular corollas. The greater number of blossoms in the control groups appeared in the 21- to 40-day period.

### Summary

Blossoms developing from plants of *Salpiglossis sinuata*, *Phlox drummondii*, and *Nicotiana tabacum* which had been irradiated during the repro-

GROUP I. RECORD OF 741 BLOSSOMS FROM FORTY-SIX PLANTS

	DAYS 1-20 SIZE OF BUD WHEN IRRADIATED				DAYS 21-40 SIZE OF BUD WHEN IRRADIATED				DAYS 41-60 SIZE OF BUD WHEN IRRADIATED				DAYS 61-80 BUDS ALL INVISIBLE	TOTAL
	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVIS- IBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVIS- IBLE	TOTAL	1 CM. OR OVER	LESS THAN 1 CM.	INVIS- IBLE		

ANOMALIES IN 555 BLOSSOMS

Number .....	129	13	114	2	465	0	210	255	171	0	8	163	5
Percentage ..	10.7	10.0	88.4	1.5	60.4	0.0	45.2	54.8	22.2	0.0	4.7	95.3	6.5

NORMAL BLOSSOMS (25.1 PER CENT. OF TOTAL)

Number .....	80	53	27	0	37	0	11	26	65	0	3	62	4
Percentage ..	43.0	66.3	33.7	0.0	19.9	0.0	29.7	70.3	34.9	0.0	4.6	95.4	2.1

GROUP II. RECORD OF 250 BLOSSOMS FROM SIXTEEN PLANTS

ANOMALIES IN 197 BLOSSOMS

Number .....	51	8	43	0	153	0	35	118	46	0	0	46	
Percentage ..	20.4	15.7	84.3	0.0	61.2	0.0	22.9	77.1	18.4	0.0	0.0	100.0	

NORMAL BLOSSOMS (21.2 PER CENT. OF TOTAL)

Number .....	15	8	7	0	26	0	20	6	12	0	0	12	
Percentage ..	28.3	53.3	46.7	0.0	49.0	0.0	76.9	23.1	22.6	0.0	0.0	100.0	



TABLE VI

RECORD FOR 454 BLOSSOMS FROM 39 CONTROL SALPIGLOSSIS PLANTS

GROUP I (327 BLOSSOMS FROM 26 PLANTS)					
	TOTAL	DAYS 1-20	DAYS 21-40	DAYS 41-60	DAYS 61-80
Number of normal blossoms	292	100	127	61	4
Percentage of total .....	89.3	34.2	43.5	20.9	1.4
Number of anomalies .....	35	0	14	20	1
Percentage of total .....	10.7	0	40	57.1	2.9
GROUP II (127 BLOSSOMS FROM 13 PLANTS)					
Number of normal blossoms	110	9	61	40	
Percentage of total .....	86.6	8.2	55.4	36.4	
Number of anomalies .....	17	0	14	3	
Percentage of total .....	13.4	0	82.3	17.6	

ductive stage with one medium dose of X-rays showed approximately 10 types of floral anomalies. The following were common to all three species: white stippling, spotting, and streaking of corolla lobes, color changes, increased and decreased number of corolla lobes, and dwarf blossoms. *Salpiglossis sinuata* was the only species of the three having puckered corolla tissue and irregularity in placement of floral organs. Cases of split corolla tube evident in *Nicotiana* and *Salpiglossis* were not seen in *Phlox*. Dissected or fimbriated margins of corolla lobes so conspicuous in *Phlox* and *Salpiglossis* were absent in *Nicotiana*.

Buds of *Phlox* and *Nicotiana* were markedly ray-susceptible, for a dose of 2,000 r-units caused frequent abscission of buds in these two species; but the same dose did not cause bud abscission in *Salpiglossis*.

A definite relationship was found between the size of the buds at the time of irradiation and the occurrence and character of the anomaly produced in the flower. From the study with *Salpiglossis* in which hundreds of buds were measured at the time of radiation, it was definitely determined that buds over one centimeter in length at the time of irradiation usually developed normally. Many of those under one centimeter in length exhibited various types of irregularities as did those forming from new growth which developed after the plants were treated. Types of anomalies generally appearing during the early part of the blooming period included stippling, spotting, and streaking of the corolla, also changes in color. Those which more commonly occurred during the middle and latter part of the flowering period were split corolla tubes, dissected margin of corolla lobes, puckered corolla tissue, increase and decrease in number of corolla lobes, and dwarf blossoms. Toward the end of the blooming period, normal blossoms began to appear in increased numbers.

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## SOME EFFECTS OF METAL SALTS ON YEAST DEHYDROGENASE

CHARLES WILLIAM HOCK

(WITH ONE FIGURE)

The results of many investigations show that solutions of electrolytes have pronounced effects upon biological systems. Permeability, viscosity, elasticity, irritability, respiration, and growth are but a few of the properties of such systems which are influenced by the presence of various anions and cations. It is now generally recognized that dehydrogenases, *i.e.*, respiratory enzymes concerned with the activation and the removal of hydrogen from the substrates which become oxidized, are universally distributed in plant and animal cells, and that they play an important rôle in normal respiration (4, 5, 6, 9). The present investigation was undertaken to determine the effects of certain representative metals on the dehydrogenase systems of yeast and of macerated yeast extract.

### Materials and method

Bakers' yeast (or extract thereof) was used for all experiments. In order to reduce to a minimum traces of heavy metals which if present might influence the reactions, all solutions were made with water which had been twice distilled. For buffering these solutions phosphates could not be used, as most of the metals under investigation form precipitates with the phosphate ion. Accordingly, a sodium acetate-acetic acid buffer mixture maintaining a pH of 6.2 was selected (11). This was made by adding one part of 0.1 M acetic acid to ten parts of 0.5 M sodium acetate.

### SUSPENSION OF YEAST CELLS

The suspension was made by mixing one part of yeast with two parts of buffer. This was done just before each trial. Tests showed that the buffer mixture was efficient in maintaining a constant pH of 6.2 throughout the course of an experiment.

### MACERATED YEAST EXTRACT

Equal amounts, by weight, of yeast and of powdered glass were ground in a mortar until a thick paste was obtained. In preliminary tests the glass powder was washed several times with distilled water to remove any soluble impurities which might influence the reaction. Since no differences could be observed between extracts prepared with washed and with unwashed glass, the practice was discontinued. There was added to the ground yeast paste twice as much buffer as yeast contained therein. This made the

amount of yeast in one volume of extract equal to that in the same volume of whole yeast suspension. After a thorough stirring the mixture was filtered through Whatman no. 1 filter paper and the extract thus obtained was kept in a cool place until needed. Since it was noted that the yeast

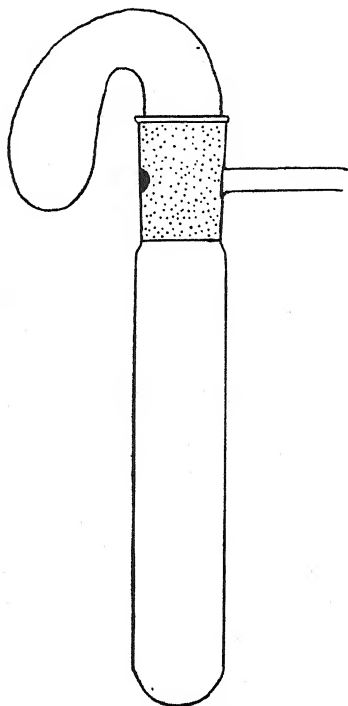


FIG. 1. THUNBERG test-tube used in the experiments reported.

extract lost some of its dehydrogenase potency upon standing, every effort was made to use the extract as soon as possible.

#### CHEMICAL REAGENTS

Solutions of the following metal salts were used in the tests:  $\text{KCl}$ ,  $\text{KNO}_3$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{HgCl}_2$ ,  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ,  $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{AuCl}_3 \cdot 2\text{H}_2\text{O}$ ,  $\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{Th}(\text{NO}_3)_4 \cdot 4\text{H}_2\text{O}$ . In every case the purest salts obtainable were employed. A 1:5000 dilution of methylene blue was used as the indicator, and sodium lactate in a final concentration of 0.02 N was added as a substrate.

#### METHOD

The simple and widely used THUNBERG technique was employed. That form of the THUNBERG test-tube with the hollow stopper (fig. 1) was selected because of its adaptability to the present investigation.

When a solution of methylene blue is added to a suspension of yeast cells in the presence of a substrate such as sodium lactate, and the whole system evacuated, a rapid reduction to colorless methylene blue results, while the lactic acid is oxidized. In such cases the dye acts as an acceptor for the two hydrogen atoms removed from the substrate. The plan adopted was to add increasing dilutions of salts to such systems and to note the effects of the metals on the rate of decolorization.

Both the yeast suspension and the yeast extract will decolorize methylene blue without the addition of a substrate, owing to the presence of oxidizable substrates normally present in the yeast itself, but the rate of the reaction is greatly accelerated by the addition of more substrate such as sodium lactate, here employed in a final concentration of 0.02 N. In all experiments each tube contained 1 ml. of methylene blue, 1 ml. 0.02 N sodium lactate, and 1 ml. of the metal salt solution. Two ml. of the yeast suspension or of the yeast extract was put in the hollow stopper. The tubes were then evacuated and allowed to come to 30° C. in a constant temperature chamber. After mixing, the time of decolorization in each case was noted. A control was run in which distilled water was substituted for the salt solution.

### Results

Table I records the time of decolorization by suspensions of yeast cells in the presence of various concentrations of the metal salts. Table III gives the results when macerated yeast extract was used. Tables II and IV record for yeast suspensions and for yeast extract, respectively, the percentage of increase or decrease in time of decolorization caused by the addition of the metals to the systems. By reducing the results of all tests to a percentage basis, individual differences among control tubes are eliminated, and the results for yeast suspensions and for yeast extract are comparable. Each reading in the tables represents an average of ten tests. Since chlorides of some metals and nitrates of others were used, solubility usually being the deciding factor, it became necessary to see whether or not these anions were significantly different in behavior. Since tests showed such differences to be negligible, we may conclude that variations in acceleration and in inhibition are due to the cations.

#### POTASSIUM CHLORIDE AND POTASSIUM NITRATE

Since only slight differences were noted between KCl and KNO<sub>3</sub> the averages for the two have been taken. All concentrations which were tried caused some inhibition when used with yeast cells, although of all the cations examined, this was the least inhibitive. At all except relatively high concentrations the inhibition was slight. When used with yeast extract the situation was somewhat different. High concentrations, from 0.2 N to

TABLE I  
METAL SALTS ADDED TO SUSPENSIONS OF YEAST CELLS

CONCENTRATION OF SALT IN SYSTEM	TIME OF DECOLORIZATION RECORDED IN MINUTES							
	KCl KNO <sub>3</sub>	CuCl <sub>2</sub>	HgCl <sub>2</sub>	MnCl <sub>2</sub>	BaCl <sub>2</sub>	AgCl <sub>3</sub>	LiA(NO <sub>3</sub> ) <sub>3</sub>	TH(NO <sub>3</sub> ) <sub>4</sub>
<i>Normality</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>	<i>min.</i>
0.4 N .....								
0.2 N .....	17.1	*	*	35.0	26.2	*		*
2.0 × 10 <sup>-1</sup> N .....	13.8	*		20.3	24.2	220.0	29.3	83.8
4.0 × 10 <sup>-2</sup> N .....		262.0	421.0	17.9	18.0	16.3	20.0	42.8
2.0 × 10 <sup>-3</sup> N .....	13.0	121.0	108.0	14.5	13.8	11.2	14.8	16.5
6.6 × 10 <sup>-3</sup> N .....		86.2	79.9		13.1	9.2	11.0	10.4
4.0 × 10 <sup>-3</sup> N .....		53.5	59.0	14.2	12.0	8.1	11.3	9.8
2.0 × 10 <sup>-3</sup> N .....	12.8	32.3	40.4	13.0	12.7	8.4	11.7	10.1
4.0 × 10 <sup>-4</sup> N .....	13.2	14.0	29.2					
2.0 × 10 <sup>-4</sup> N .....	13.1	13.3	17.4	12.9	12.0	8.3	11.4	10.6
2.0 × 10 <sup>-5</sup> N .....	12.4	13.6	16.8	12.6	12.8	8.8	11.2	10.4
2.0 × 10 <sup>-6</sup> N .....	12.9	13.8	17.1	12.4	11.6	8.6	10.8	10.6
2.0 × 10 <sup>-7</sup> N .....	12.6	14.0		12.4	10.9	8.8	10.6	10.6
2.0 × 10 <sup>-8</sup> N .....		14.3		12.4			10.6	
2.0 × 10 <sup>-9</sup> N .....		14.5		12.6			10.4	
2.0 × 10 <sup>-10</sup> N .....		15.3		12.2				
2.0 × 10 <sup>-11</sup> N .....		14.7		12.6				
2.0 × 10 <sup>-12</sup> N .....		14.7		13.1				
2.0 × 10 <sup>-13</sup> N .....		14.7		13.1				
Control .....	12.5	14.5	16.7	13.1	10.9	8.6	10.4	10.6

\* No decolorization in 12 hours.

TABLE II  
METAL SALTS ADDED TO SUSPENSIONS OF YEAST CELLS

CONCENTRATION OF SALT IN SYSTEM	PERCENTAGE INCREASE OR DECREASE IN TIME OF DECOLORIZATION CAUSED BY METALS							
	KCl KNO <sub>3</sub>	CuCl <sub>2</sub>	HgCl <sub>2</sub>	MnCl <sub>2</sub>	BaCl <sub>2</sub>	AuCl <sub>3</sub>	La(NO <sub>3</sub> ) <sub>3</sub>	Th(NO <sub>3</sub> ) <sub>4</sub>
Normality	%	%	%	%	%	%	%	%
0.4 N	+37.0	*	*	+160.0	+140.0	*		*
0.2 N	+10.0	*	*	+55.0	+120.0	+2400.0	+180.0	+690.0
2.0 × 10 <sup>-1</sup> N				+35.0	+65.0	+45.0	+90.0	+300.0
4.0 × 10 <sup>-2</sup> N	+4.0	+1700.0	+2400.0	+10.0	+25.0	+30.0	+40.0	+55.0
2.0 × 10 <sup>-2</sup> N		+780.0	+550.0		+20.0	+6.9	+5.7	1.9
6.6 × 10 <sup>-3</sup> N		+490.0	+380.0	8.4	+10.0	5.8	8.6	7.5
4.0 × 10 <sup>-3</sup> N		+270.0	+250.0	0.7	+15.0	2.1	10.0	4.7
2.0 × 10 <sup>-3</sup> N	+4.8	+120.0	+140.0					
4.0 × 10 <sup>-4</sup> N		-3.4	+75.0					
2.0 × 10 <sup>-4</sup> N	+2.4	-8.2	+4.1	1.5	+10.0	-3.4	+9.6	0.0
2.0 × 10 <sup>-5</sup> N	+5.6	-6.2	+0.6	3.8	+15.0	+2.1	+7.7	1.9
2.0 × 10 <sup>-6</sup> N	+4.8	-4.1	+0.6	5.3	+6.4	0.0	+3.8	0.0
2.0 × 10 <sup>-7</sup> N	-0.8	-3.4	+2.4	5.3	0.0	+2.1	+1.9	0.0
2.0 × 10 <sup>-8</sup> N	+3.2	-1.3		5.3			+1.9	
2.0 × 10 <sup>-9</sup> N	+0.8	0.0		3.8			0.0	
2.0 × 10 <sup>-10</sup> N		+5.5		6.8				
2.0 × 10 <sup>-11</sup> N		+1.3		3.8				
2.0 × 10 <sup>-12</sup> N		+1.3		0.0				
2.0 × 10 <sup>-13</sup> N		+1.3		0.0				

\* No decolorization in 12 hours.

TABLE III  
METAL SALTS ADDED TO MACERATED YEAST EXTRACT

CONCENTRATION OF SALT IN SYSTEM	TIME OF DECOLORIZATION RECORDED IN MINUTES							
	KCl KNO <sub>3</sub>	CuCl <sub>2</sub>	HgCl <sub>2</sub>	MnCl <sub>2</sub>	BaCl <sub>2</sub>	AuCl <sub>3</sub>	La(NO <sub>3</sub> ) <sub>3</sub>	Th(NO <sub>3</sub> ) <sub>4</sub>
Normality	min.	min.	min.	min.	min.	min.	min.	min.
0.4 N .....	2.2	*	*	1.4	1.5	*	5.5	*
0.2 N .....	2.4	*	*	1.5	1.7	48.0	4.1	10.4
2.0 × 10 <sup>-1</sup> N .....	2.5	535.0	244.0	2.5	2.1	4.4	2.7	3.3
4.0 × 10 <sup>-2</sup> N .....		250.0	231.0	2.7	2.4	4.3	2.8	3.2
2.0 × 10 <sup>-2</sup> N .....		56.4	101.0		2.8	3.4	2.8	3.5
6.6 × 10 <sup>-3</sup> N .....		19.8	56.9	3.0	2.9	3.2	2.7	4.2
4.0 × 10 <sup>-3</sup> N .....	2.6	7.7	8.4	2.9	2.7	3.9	2.4	4.3
2.0 × 10 <sup>-3</sup> N .....		6.1	5.6		2.7		3.0	4.4
4.0 × 10 <sup>-4</sup> N .....	2.7	5.4	4.3	2.9	2.6	3.8	2.7	4.4
2.0 × 10 <sup>-5</sup> N .....	2.8	5.5	4.2	2.6	2.7	3.9	2.5	4.3
2.0 × 10 <sup>-6</sup> N .....	2.7	5.2	4.4	2.7	2.5	3.9	2.7	4.5
2.0 × 10 <sup>-7</sup> N .....	2.8	5.8	4.0	2.8			2.4	
2.0 × 10 <sup>-8</sup> N .....	2.7	5.7	3.8	2.6			2.4	
2.0 × 10 <sup>-9</sup> N .....		5.7	4.6	2.6				
2.0 × 10 <sup>-10</sup> N .....		5.7		2.6				
2.0 × 10 <sup>-11</sup> N .....		5.8		2.6				
2.0 × 10 <sup>-12</sup> N .....		6.0		2.6				
2.0 × 10 <sup>-13</sup> N .....		5.7		2.9				
Control .....	2.7	5.7	4.6	2.9	2.5	3.5	2.5	4.2

\* No decolorization in 12 hours.



TABLE IV  
METAL SALTS ADDED TO MACERATED YEAST EXTRACT

CONCENTRATION OF SALT IN SYSTEM	PERCENTAGE INCREASE OR DECREASE IN TIME OF DECOLORIZATION CAUSED BY METALS							
	KCl KNO <sub>3</sub>	CuCl <sub>2</sub>	HgCl <sub>2</sub>	MnCl <sub>2</sub>	BaCl <sub>2</sub>	AuCl <sub>3</sub>	LiA(NO <sub>3</sub> ) <sub>3</sub>	TH(NO <sub>3</sub> ) <sub>4</sub>
Normality	%	%	%	%	%	%	%	%
0.4 N	-19.0	*	*	-52.0	-40.0	*		*
0.2 N	-11.0	*	*	-48.0	-32.0		+64.0	+150.0
2.0 × 10 <sup>-1</sup> N				-14.0	-16.0	+1300.0	+8.0	-20.0
4.0 × 10 <sup>-2</sup> N				-6.9	-4.0	+25.0	+12.0	-23.0
2.0 × 10 <sup>-3</sup> N	-7.4	+9300.0	+5200.0		+12.0	+23.0	+12.0	-16.0
6.6 × 10 <sup>-3</sup> N		+890.0	+4900.0	(?) + 3.4	+12.0	-8.5	+8.0	0.0
2.0 × 10 <sup>-3</sup> N		+250.0	+2900.0	(?) 0.0	+16.0	+11.5	+8.0	+2.3
4.0 × 10 <sup>-3</sup> N	-3.7	+35.0	+1100.0		+8.0			
2.0 × 10 <sup>-4</sup> N		+7.4	+85.0		+4.0	+8.6	+20.0	+4.7
2.0 × 10 <sup>-4</sup> N	0.0	-2.1	+20.0	0.0	+8.0	+11.0	+8.0	+4.7
2.0 × 10 <sup>-5</sup> N	+3.7	-2.1	-6.5	0.0	0.0	+8.6	0.0	+2.3
2.0 × 10 <sup>-6</sup> N	0.0	-1.3	-8.7	-10.0	+8.0		+8.0	+7.1
2.0 × 10 <sup>-7</sup> N	+3.7	-8.7	-4.3	-6.9	0.0			
2.0 × 10 <sup>-8</sup> N	+3.7	+1.7	+13.0	-3.4		+11.0		
2.0 × 10 <sup>-9</sup> N	0.0	0.0	+17.0	-10.0				
2.0 × 10 <sup>-10</sup> N		0.0	0.0	-10.0				
2.0 × 10 <sup>-11</sup> N		+1.7		-10.0				
2.0 × 10 <sup>-12</sup> N		+2.1		-10.0				
2.0 × 10 <sup>-13</sup> N		0.0		0.0				

\* No decolorization in 12 hours.

$2.0 \times 10^{-3}$  N, increased the velocity of decolorization. Lower concentrations were without effect or very slightly inhibitive. FLEISCHMANN and SCHWARZ (2), using yeast cells, found that sodium accelerated the dehydrogenation of sodium succinate whereas potassium inhibited the oxidation. They observed no differences between these two ions when added to macerated yeast extract.

#### CUPRIC CHLORIDE

When  $\text{CuCl}_2$  is present in a concentration of 0.02 N or greater the dehydrogenase activity of yeast cells and of extract is completely inhibited. In concentrations ranging from  $4.0 \times 10^{-4}$  N to  $2.0 \times 10^{-3}$  N, inclusive, the rate of decolorization of methylene blue by yeast cells is increased; concentrations from  $2.0 \times 10^{-4}$  to  $2.0 \times 10^{-7}$  N have a slightly accelerating effect when yeast extract is used.

#### MERCURIC CHLORIDE

All concentrations of this salt exerted an inhibitive action on a suspension of yeast cells. No decolorization by yeast cells was obtained when concentrations 0.02 N or greater were used. The mercuric ion accelerated the dehydrogenase activity of extract when present in concentrations from  $2.0 \times 10^{-5}$  N to  $2.0 \times 10^{-9}$  N, inclusive. Concentrations of greater normality were inhibitive.

#### MANGANESE CHLORIDE

Down to a concentration of  $4.0 \times 10^{-3}$  N this cation exerted inhibitive action on suspensions of yeast cells. At greater dilutions, as great as  $2.0 \times 10^{-11}$  N, the presence of the metal hastened decolorization of the dye, *i.e.*, the metal promoted dehydrogenase activity. The effect of this metal on yeast extract was remarkable. All concentrations from 0.2 N to  $2.0 \times 10^{-12}$  N seemed able to reduce the time of decolorization.

#### BARIUM CHLORIDE

Concentrations ranging from 0.4 N to  $2.0 \times 10^{-6}$  N retarded slightly the decolorization of methylene blue by the dehydrogenase of yeast cells. Low concentrations of the metal were weakly toxic to yeast extract. The dehydrogenation of lactic acid by extract was definitely accelerated by higher concentrations of  $\text{BaCl}_2$ , as much as 40 per cent. when barium was present in a 0.4 N concentration. Although barium exerted a remarkable promoter action, this was evident only in high concentrations— $4.0 \times 10^{-2}$  N and greater.

#### AURIC CHLORIDE

At concentrations between  $4.0 \times 10^{-3}$  N and  $2.0 \times 10^{-4}$  N, inclusive, the gold ion increased the velocity of reaction with suspensions of yeast cells.



With extract the range of stimulation is narrower, from  $6.6 \times 10^{-3}$  N to  $4.0 \times 10^{-3}$  N. In both cases the addition of normal solutions, or of greater concentrations, completely inhibit dehydrogenase catalyses.

#### LANTHANUM NITRATE

All concentrations of lanthanum from  $2.0 \times 10^{-1}$  N to  $2.0 \times 10^{-8}$  N increase the time of decolorization of methylene blue by yeast cells and by yeast extract. The inhibition is appreciable, however, only in concentrations of  $2.0 \times 10^{-2}$  N and greater.

#### THORIUM NITRATE

Dehydrogenase activity is reduced to zero by concentrations of thorium 0.2 N or greater. With a suspension of yeast cells this metal ion is stimulative in concentrations from  $6.6 \times 10^{-3}$  N to  $2.0 \times 10^{-3}$  N, or possibly  $2.0 \times 10^{-5}$  N (see table II). Greater amounts of the metal must be present in order to stimulate yeast extract, *i.e.*, concentrations from  $4.0 \times 10^{-2}$  to  $6.6 \times 10^{-3}$  N, but the stimulation, once induced, speeds up the reaction three or four times more than with yeast cells.

#### Discussion

Certain differences were regularly observed between the behavior of yeast suspensions and yeast extract. After the dye had been decolorized anaerobically with extract, the blue color could be restored only by prolonged and vigorous shaking in air. This may mean that the leucomethylene blue is adsorbed in some way which renders it inaccessible, possibly by occlusion to the maceration material. The autoxidation of leucomethylene blue to methylene blue proceeds less rapidly in control tubes of extract than in those containing the metals.

The decolorization by yeast extract is approximately three and one-half times more rapid than by yeast cells. Undoubtedly this means that when yeast cells are used, permeability is a factor of considerable importance. Grinding and extracting make the colloidal enzyme surfaces immediately available. However, when time of decolorization is plotted against the concentration of the metal added to the system, the resulting curves are similar whether yeast suspension or yeast extract has been used. This indicates that the effects of the ions on suspensions as well as on extracts is on the enzyme reactivity rather than on permeability.

Several experimental observations pointed to the association of inhibition with colloidal phenomena, especially adsorption. Metals manifest their inhibitive effects no matter when they are added to the dehydrogenase systems. Suppose a control tube, *i.e.*, one containing no metal, is decolorized. If, then, an inhibitive concentration of metal is added and decolorization allowed to proceed a second time, the formation of leucomethylene blue is

retarded. Presumably, metals inhibit dehydrogenase activity by diminishing the active catalyst surface.

The slight acceleration by traces of salts is not easily explained. It might be that small amounts of some of the added electrolytes exert an effect on the dispersion of the colloidal components of the cells and of the yeast extract. On the other hand, dilute solutions of some of the electrolytes might react selectively with the prosthetic group of the enzyme, whereas larger amounts exert a "blanket" effect on the enzyme carrier. This latter explanation, however, would not hold in all cases inasmuch as Ba, Mn, and K caused the greatest acceleration when present in high concentrations.

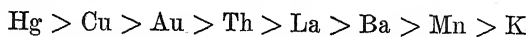
THUNBERG (7, 8) observed the effects of metallic salts on the rate of decolorization of indicators by oxidation-reduction systems of plant seeds. He found that low concentrations of Hg, Cu, and Ag accelerated, whereas high concentrations inhibited the decolorization of dyes by extracts of the seeds of *Trifolium pratense*. With other seeds on which Zn and Cd exerted a stimulative effect Ag had an indefinite action.

That a small fraction of a fatal concentration of certain metals may be used as a stimulant is an established physiological principle and has been observed by many investigators. Increasing concentrations of the metal become toxic. COPELAND (1) experimented with *Elodea*, *Callitriche*, a crucifer, a fish, and frog larvae, and found that small amounts of Cu, Zn, Cd, Ag, and Hg, stimulated respiration as measured by carbon dioxide evolution. VAN LAER (10) reached similar conclusions using yeast cells. LE VAN (3) found that low concentrations of Cu, Cd, Al, Na, Mg, and Fe increased respiration in *Lupinus albus*. The present investigation gives evidence that the stimulation which certain metals produce at low concentrations, and the inhibition which they produce at high concentrations, may be caused by a specific effect which these metals have on the dehydrogenase systems of the cells.

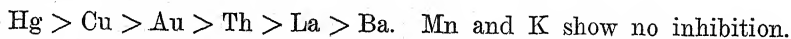
### Summary

1. Using the methylene blue technique of THUNBERG the effects of KCl, KNO<sub>3</sub>, CuCl<sub>2</sub>, MnCl<sub>2</sub>, BaCl<sub>2</sub>, AuCl<sub>3</sub>, La(NO<sub>3</sub>)<sub>3</sub>, Th(NO<sub>3</sub>)<sub>4</sub> on the dehydrogenase of yeast and of macerated yeast extract were noted.

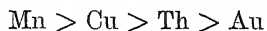
2. The order in which the cations inhibit the dehydrogenase activity of yeast cells is as follows:



3. The cations are inhibitive to the dehydrogenase system of yeast extract in the following order:



4. At low concentrations four of the cations accelerate slightly when yeast cells are used.



5. With yeast extract Mn, Ba, and K accelerate dehydrogenase action markedly, especially in high concentrations. Th, Hg, Cu, and Au cause a slight acceleration in low concentrations, but inhibit strongly in high concentrations. Both high and low concentrations of Mn promote the catalytic activity of the extract. La in low concentrations has no effect, but inhibits in high concentrations.

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## PECTIC CONTENT OF PLANT MATERIALS

WILLIAM E. ELWELL AND WILLIAM M. DEHN

Many plants and plant materials such as wood (2), tea (25), tobacco (15), cotton (16), hops (13), ramie (11), flax (12), grass (6), potato (10), plantain seed (20), celery (9), limes (22), guava (1), bergamot (23), and root hairs (21), have been shown to contain pectic compounds. Because different methods of extraction and analysis were employed, it is impossible to make comparative studies from these data of the pectic composition, types of pectic materials, and distribution of pectic compounds in these plants. A systematic study, employing uniform treatment of the pectic materials of leaves, cereal grains, apples, and citrus fruits, was conducted by NANJİ and NORMAN (18). They determined water-soluble pectin, oxalic-acid-soluble pectin and ammonium-oxalate-soluble pectin. These data were interpreted as representing pectic material present as pectin, pectin in combination with metallic ions, and pectic acid. This interpretation was based on the theory that protopectin is an insoluble calcium, magnesium, or iron salt (19), and that treatment with oxalic acid and ammonium oxalate dissolved the pectin and pectates combined in this manner. More recent studies, however, (3, 4, 14, 17, 24), lead to the conclusion that the pectic molecule is essentially a long-chain, poly-galacturonic molecule combined through intermolecular forces and anhydride structure to form the insoluble protopectin layers of the cell wall and middle lamella. Single treatment with water or acid does not remove any definite pectic compound, but yields a part of a series of pectic substances; continued hydrolysis progressively dissolves the entire protopectin layer.

The lack of quantitative data for comparison of the distribution of pectin in the plant phyla has led to the present study. A large number of plants and plant materials have been included, and in order to obtain comparative data for the concentrations of each pectic fraction present, a system of extraction involving successive stages of hydrolysis was employed. The first stage involves the removal of the water soluble pectin; the second, the easily hydrolyzable protopectin; and the third, the difficultly hydrolyzable protopectin. These analyses have been supplemented by data on gelling power of the pectic material.

### Extraction and analyses

One hundred grams of the material was ground in a sausage mill; 500 ml. of water were added, and the mixture was heated for one hour at 90° C., evaporated water being replaced during the heating. The pulp was then pressed free of the liquid and returned for a second extraction with 500 ml. of water containing 0.155 ml.  $\text{H}_2\text{SO}_4$  (pH = 1.5). The pulp was again

TABLE I

PECTIN CONTENT OF VARIOUS PLANT TISSUES

PLANT	EXTRACT NO.	PECTIN AS CALCIUM PECTATE		PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	WET BASIS	DRY BASIS	
Apple ( <i>Pyrus malus</i> ) .....	1	%	%	%	%	Good
	2	0.37	2.46	0.22	1.36	
	3	1.00	6.67	0.21	1.35	
	3	0.23	1.37	0.18	1.20	
	Total	1.60	10.50	0.61	3.91	
Carrot ( <i>Daucus carota</i> ) ...	1	0.97	10.80	0.64	7.12	None
	2	0.33	3.68	0.62	6.89	
	3	0.21	2.34	0.41	4.56	
	Total	1.51	16.82	1.67	18.75	
Cranberry ( <i>Vaccinium macrocarpon</i> ) .....	1	0.31	2.21	0.38	2.71	Some
	2	0.34	1.64	0.22	1.57	
	3	0.04	2.85	0.12	0.86	
	Total	0.69	6.70	0.72	5.14	
Fern ( <i>Pteridium aquilinum</i> var. <i>pubescens</i> Underwood) .....	1	0.57	1.78	0.17	0.53	Good
	2	0.37	1.16	0.76	2.37	
	3	0.21	0.61	0.26	0.81	
	Total	1.15	3.55	1.19	3.71	
Grape ( <i>Vitis vinifera</i> ) .....	1	0.34	1.36	0.48	1.92	Some
	2	0.96	3.84	0.55	2.40	
	3	0.38	1.52	0.37	1.48	
	Total	1.68	6.72	1.40	5.80	
High bush cranberry (berry) ( <i>Viburnum opulus</i> ) .....	1	2.45	14.0	0.16	0.91	Good
	2	2.82	16.1	0.32	1.83	
	3	3.59	20.5	.....	.....	
	Total	8.86	50.6	0.48	2.74	
High bush cranberry (stored 3 months at 10° C.) .....	1	1.08	6.18	0.71	4.06	Slight
	2	1.28	7.32	0.62	3.54	
	3	0.53	3.03	0.38	2.17	
	Total	2.89	16.53	1.71	9.77	
Mountain ash (berry) ( <i>Pyrus americana</i> ) .....	1	0.90	2.57	0.22	0.63	Good
	2	0.96	2.74	0.98	2.79	
	3	0.88	2.51	.....	.....	
	Total	2.74	7.82	1.20	3.42	
Mountain ash (stored 3 months at 10° C.) .....	1	0.38	1.09	0.39	1.11	Slight
	2	0.19	0.54	0.49	1.40	
	3	0.01	0.03	0.21	0.60	
	Total	0.58	1.66	1.09	3.11	



TABLE I (Continued)  
PECTIC CONTENT OF VARIOUS PLANT TISSUES

PLANT	EXTRACT NO.	PECTIN AS CALCIUM PECTATE		PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	WET BASIS	DRY BASIS	
Pea (hull) ( <i>Pisum sativum</i> ) .....	1	%	%	%	%	Good
	2	0.45	3.21	0.08	0.57	
	3	0.67	4.78	0.81	5.78	
	3	0.11	0.79	0.08	0.57	
Total		1.23	8.78	0.97	6.92	
Scotch broom ( <i>Cytisus scoparius</i> ) .....	1	0.14	0.35	0.12	0.30	Good
	2	1.16	2.90	0.57	1.43	
	3	0.68	1.70	0.43	1.07	
	3	0.68	1.70	0.43	1.07	
Total		1.98	4.95	1.12	2.80	
Snow berry (berry) ( <i>Symphoricarpos racemosus</i> )	1	0.27	1.69	0.01	0.06	Some
	2	0.53	3.31	0.41	1.60	
	3	1.50	9.37	1.00	6.25	
	3	1.50	9.37	1.00	6.25	
Total		2.30	14.37	1.42	7.91	
Sow thistle ( <i>Sonchus oleraceus</i> ) .....	1	0.37	2.18	0.07	0.41	Good
	2	0.72	4.24	0.42	2.47	
	3	0.35	2.06	0.18	1.06	
	3	0.35	2.06	0.18	1.06	
Total		1.44	8.48	0.67	3.94	
St. John's wort (bud) ( <i>Hypericum calycinum</i> )	1	1.10	4.08	0.13	4.82	Good
	2	2.69	9.62	5.03	18.60	
	3	1.17	4.34	0.69	2.56	
	3	1.17	4.34	0.69	2.56	
Total		4.96	18.04	5.85	25.98	
St. John's wort (stem) .....	1	0.83	1.93	0.06	0.14	Good
	2	1.18	2.75	2.24	7.27	
	3	0.92	2.14	0.54	1.25	
	3	0.92	2.14	0.54	1.25	
Total		2.93	6.82	2.84	8.66	

pressed and returned for a third extraction; the same quantity of the acid solution previously used was added and a 2-hr. extraction was employed. The filtrates obtained from the three extractions were each made up to one liter, and aliquot parts were used for analysis. Data on the pectin content of various plants, as determined both by the method of calcium pectate precipitation (22) and by the method of alcohol precipitation, are given in table I, with calculations to the dry basis. Data on the pectin content of vegetables, obtained by the method of alcohol precipitation, are shown in table II with calculations to the dry basis. Data on the pectin content of apples according to this method of treatment are included in table I to serve for comparison with other published analyses.

TABLE II  
PECTIC CONTENT OF VARIOUS VEGETABLES

PLANT	EXTRACT NO.	PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	
		%	%	
Artichoke ( <i>Cynara scolymus</i> ) .....	1	1.28	7.12	Slight
	2	0.87	4.83	
	3	0.34	1.89	
	Total	2.49	13.84	
Asparagus ( <i>Asparagus officinalis</i> ) .....	1	0.29	4.15	None
	2	1.56	22.10	
	3	0.16	2.29	
	Total	2.01	28.54	
Beet (top) ( <i>Beta vulgaris</i> ) .....	1	0.32	6.40	None
	2	0.43	8.63	
	3	0.41	8.20	
	Total	1.16	23.23	
Beet (root) .....	1	0.32	2.00	None
	2	0.47	2.94	
	3	0.39	2.44	
	Total	1.18	7.38	
Broccoli ( <i>Brassica oleracea</i> ) .....	1	0.84	6.46	None
	2	1.07	8.15	
	3	0.54	4.16	
	Total	2.45	18.77	
Brussels sprout ( <i>Brassica oleracea</i> L. var. <i>gemmifera</i> Zenker)	1	0.69	3.83	None
	2	0.94	5.22	
	3	0.81	4.50	
	Total	2.44	13.55	
Celery ( <i>Apium graveolens</i> ) .....	1	0.40	5.72	None
	2	0.44	6.28	
	3	0.15	2.14	
	Total	0.99	14.14	
Dandelion ( <i>Taraxacum officinalis</i> ) .....	1	2.11	8.80	None
	2	2.21	9.22	
	3	3.15	13.10	
	Total	7.47	31.42	
Lettuce ( <i>Lactuca sativa</i> ) .....	1	0.13	4.33	Slight
	2	0.28	9.34	
	3	0.24	11.32	
	Total	0.75	24.99	
Onion ( <i>Allium cepa</i> ) .....	1	0.36	3.00	None
	2	0.72	6.00	
	3	0.32	2.67	
	Total	1.40	11.67	



TABLE II (Continued)  
PECTIC CONTENT OF VARIOUS VEGETABLES

PLANT	EXTRACT NO.	PECTIN AS ALCOHOL PRECIPITATE		GELLING PROPERTIES
		WET BASIS	DRY BASIS	
		%	%	
Parsnip ( <i>Pastinaca sativa</i> ) .....	1	0.83	5.22	None
	2	1.10	6.88	
	3	1.20	7.50	
	Total	3.13	19.60	
Potato ( <i>Solanum tuberosum</i> ) .....	1	0.81	3.52	None
	2	1.17	5.08	
	3	0.77	3.35	
	Total	2.75	11.95	
Rhubarb ( <i>Rheum rhaponticum</i> )	1	2.42	30.4	Slight
	2	2.84	35.5	
	3	2.00	25.0	
	Total	7.26	90.9	
Rutabaga ( <i>Brassica napobrassica</i> Mill.) .....	1	0.39	2.17	None
	2	0.71	3.97	
	3	0.47	2.61	
	Total	1.57	8.75	
Spinach ( <i>Spinacea oleracea</i> L.)	1	0.96	3.00	None
	2	0.47	3.91	
	3	0.61	5.08	
	Total	2.04	11.99	
Squash ( <i>Cucurbita moschata</i> ) .....	1	0.20	1.82	None
	2	0.50	4.54	
	3	0.80	7.28	
	Total	1.50	13.64	
Sweet potato ( <i>Ipomoea batatas</i> )	1	1.63	4.80	None
	2	0.99	2.91	
	3	0.95	2.79	
	Total	3.57	10.50	
Swiss chard ( <i>Beta vulgaris</i> var. <i>ciela</i> L.) .....	1	0.22	4.40	None
	2	3.17	63.40	
	3	0.15	3.00	
	Total	3.54	70.80	
Tomato ( <i>Lycopersicum esculentum</i> ) .....	1	0.32	5.33	None
	2	0.19	3.17	
	3	0.11	1.84	
	Total	0.62	10.34	
Turnip ( <i>Brassica campestris</i> var. <i>turnip</i> ) .....	1	0.44	3.38	None
	2	0.39	3.00	
	3	0.44	3.38	
	Total	1.27	9.76	

Tests of the gelling power of the alcohol precipitate were made as follows: The filtrate from an one-hour extraction of 100 gm. of the material with 500 ml. of water containing 0.155 ml.  $\text{H}_2\text{SO}_4$  was added to two volumes of 95 per cent. alcohol. The precipitated pectin was separated, dried, and one gram of it with one gram tartaric acid was dissolved in 55 ml. of water, and 100 gm. of sucrose added. The mixture was boiled until a weight of 150 gm. was attained. The strength of the jelly was estimated after standing for 24 hours.

### Discussion

The data of tables I and II disclose several interesting points concerning concentration, properties, and changes of pectin in plant materials.

Among plants of the same family or genus there is no apparent correlation in amount of the three types of pectic material. In the case of *Pyrus malus* the predominant type is that obtained by hot water extraction, while *Pyrus americana* shows approximately equal concentrations of each type. Certain correlation is exhibited by the members of the genus *Brassica* in the predominance of the hot water soluble pectin.

All the plants studied are rapidly growing and BUSTON'S (5) hypothesis that pectic materials develop under conditions of rapid growth is confirmed. The table shows that slower growing, woody plants, such as Scotch broom, sow thistle, and bracken fern contain but small quantities of pectic material while the fast growing vegetables contain large quantities. On the other hand, it is evident that conditions of high pectin content and fast growth do not favor the formation of the gelling type of pectin. Plants that possess pectin of high gelling power usually contain less than 10 per cent. of pectin on the dry basis, while those of low gelling power are of the vegetable type and contain more than 10 per cent. pectin.

The plant pericarps, such as those of fruits and berries, are the usual source of gelling pectin. It is shown that stems and branches of plants may also yield pectin of the gelling type, as in the cases of Scotch broom, bracken fern, sow thistle, and St. John's wort. Other sources of gelling pectin are mountain ash, high bush cranberry, cranberry, snow berry, St. John's wort buds, and pea-hulls.

The natural process of pectin alteration in plants follows a course of protopectin hydrolysis followed by hydrolysis of soluble pectic compounds and pectates (7, 8). From data concerning the change of pectic material during storage in mountain ash, and high bush cranberry, it is concluded that during ripening the type of pectin that is obtained by the third extraction undergoes the most rapid change of the three types of pectic compounds. There is an accompanying decrease in amount of the pectic material of the types derived by the first and second extraction processes. The rate of decrease in these types is less, indicating that the enzymatic processes in-

volved in the degradation of the difficultly soluble type of pectic material proceed more rapidly than those that effect the soluble type. This may be attributed to different enzymatic actions, or, to a single process, the rate of which changes with altering conditions.

### Summary

Comparative quantitative data on the pectic content of many plant materials have been obtained; also, data indicating the enzyme processes involved in the degradation of pectin.

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# PRODUCTION AND CONSUMPTION OF ETHYLENE BY ETHYLENE-TREATED BANANAS

R. C. NELSON  
(WITH TWO FIGURES)

## Introduction

In an earlier paper (2) the author has described the emission of ethylene by bananas into a closed chamber during the ripening process. The effect of added ethylene on this process is herein described.

## Materials and methods

Bananas were ripened under the same conditions as in the earlier experiments. They were taken off the car as they arrived green in Minneapolis, and the individual "fingers" were separated from the hands and washed. 4.46 kg. of fruit were arranged on a wire stand over which was placed a bell-jar with a capacity of 14.8 liters. Within the bell-jar was a vessel holding 100 ml. of 7.5 N sodium hydroxide solution to absorb any carbon dioxide produced. The carbon dioxide absorbed was replaced by oxygen under low constant pressure from a gasometer, thus maintaining approximately normal atmospheric conditions under the bell-jar while permitting the collection of emanations. The whole was kept in a room where the temperature varied from 18° C. to 19° C. At the end of each day the fruits were uncovered and allowed to air for about an hour.

At the beginning of each day's run, immediately after the bananas had been enclosed in the bell-jar, cylinder ethylene of anaesthetic grade was measured into the jar in quantities ranging from 1.15 to 1.25 ml., using a micro gas burette. At the end of two hours, which time was found ample to permit diffusion of ethylene throughout the bell-jar, a 330-ml. sample of gas was removed from the bell-jar and its ethylene content determined. This value was taken as the initial concentration of ethylene in the atmosphere surrounding the bananas. The final concentration was determined the next day on a similar gas sample taken just before the bell-jar was opened to air the bananas.

Ethylene determinations were made according to the author's method (2). The carbon dioxide production of the bananas was measured by diluting the sodium hydroxide which had been placed in the bell-jar to 1000 ml., and titrating a 25-ml. aliquot with 0.5 N hydrochloric acid after the addition of sufficient barium chloride solution to precipitate the carbonate.

The volume of the bananas was used in certain calculations. This value, calculated from the specific gravity determined by the conventional water-displacement method on whole fruits, was found to be approximately 0.945.

The bell-jar was tested for its ability to retain ethylene. To it was added 1.35 ml. of ethylene. A sample was withdrawn after two hours; and when an ethylene determination was made on it, it was found to titrate 0.81 ml. of 0.002 N potassium permanganate solution. Twenty hours later the titre of a sample was 0.80 ml. The titre required by calculation from the first day's determination was 0.79 ml. Evidently losses of ethylene from the bell-jar may be disregarded.

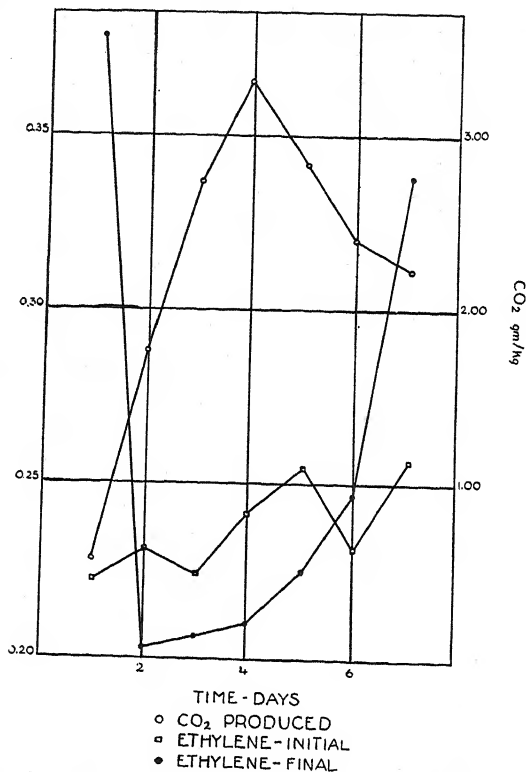


FIG. 1. Respiration and ethylene production by ripening bananas.

### Results

The results obtained are shown graphically in figure 1. It will be observed that the bananas were received just as they entered the climacteric period. At this time they were grass-green, greener than bananas are usually received at Minneapolis. By the end of the fourth day the green color had almost entirely disappeared. At the end of the fifth day the characteristic full aroma of ripe bananas was present, although the fruits were still quite firm, and even on the sixth day they had not reached the softness characteristic of fully ripe bananas.



The respiration increased rapidly to a maximum on the fourth day, after which it declined at a somewhat slower rate.

On the first day the bananas emitted about 0.16 mg. of ethylene per kg. into the air of the bell-jar where the initial concentration of ethylene was 73 p.p.m., which indicated a high concentration of ethylene in the interior of the fruit. On the second day and on the three days thereafter the movement of ethylene was in the opposite direction. On the sixth and seventh days ethylene was again emitted into the air of the bell-jar.

In table I is presented the analytical data for the initial concentrations of ethylene in the bell-jar together with the doses of ethylene applied.

TABLE I

DAY	VOLUME OF AIR	TITRATION KMnO <sub>4</sub>	C <sub>2</sub> H <sub>4</sub> DETERMINED	C <sub>2</sub> H <sub>4</sub> ADDED	PERCENTAGE RECOVERY
	l.	ml.	mg.	mg.	%
1 .....	10.1	0.99	0.99	1.47	67.0
2 .....	10.1	1.03	1.03	1.51	68.1
3 .....	10.1	1.00	1.00	1.47	67.7
4 .....	10.5	0.94	0.975	1.42	68.5
5 .....	10.5	0.99	1.03	1.49	69.0
6 .....	10.5	0.90	0.935	1.45	64.8
7 .....	10.6	0.96	1.005	1.51	66.8
8 .....	14.8	0.81	1.19	1.69	71.0

The value "mg. ethylene added" was calculated from a volume which had not been corrected for temperature and pressure, and the actual percentages of recovery would be about 10 per cent. higher. The reason for the low recovery shown in the last column of table I is not certain, but it is prob-

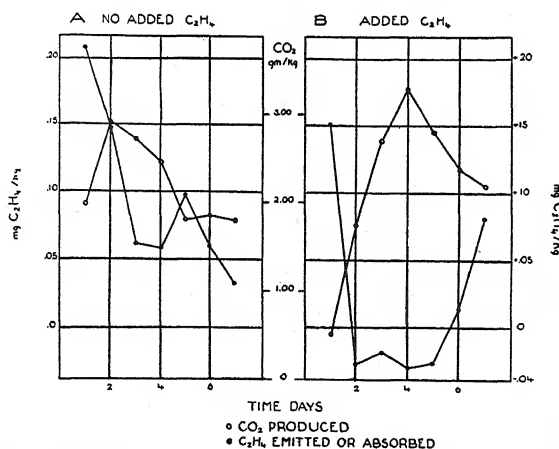


FIG. 2. Respiration and ethylene exchange of ripening bananas in the presence and absence of added ethylene.

ably caused by adsorption of ethylene on the walls of the bell-jar. The relatively large surface of the jar and the small quantity of ethylene added make this explanation plausible. When ten times the quantity of ethylene is added, and a smaller sample taken the recovery is almost quantitative. Evidently about as much ethylene is lost during airing as is adsorbed during the two hours which elapse between giving the dose of ethylene and withdrawing a sample for determination. The overnight leakage trial showed that no ethylene was lost from the atmosphere after the first two hours.

The adsorption of the odorous constituents of banana on the bell-jar is easily demonstrated. If after airing until no further aroma of banana can be detected in the jar, it is closed again, apart from the bananas, after a short time the banana odor may be detected in it.

### Discussion

Figure 2 shows a comparison of runs made on bananas under similar conditions with and without added ethylene. In an earlier paper (2) the author suggested that the rise in ethylene emission on the fifth day (fig. 2A), coincident with the end of the ripening process, might be an indication that ethylene is consumed in the ripening process in banana. The results obtained in the present experiment seem to support this view.

A more complete demonstration of the consumption of ethylene might have been obtained by determining the residual ethylene present in the bananas. The extent of loss of ethylene entailed in the sampling process, however, is not known and it was felt that the significance of such analyses might not be great. Furthermore, this procedure would cause a considerable daily decrease in the banana tissue beneath the bell-jar, which is undesirable.

Some ethylene is lost from the fruits during the 1-hour airing period. A comparison, however, of the percentage recovery of ethylene in control determinations with that in determinations with bananas in the bell-jar indicates that only about 3 per cent. of the ethylene dose penetrates into the bananas in the 2-hour period allowed for diffusion. It seems likely that the loss during airing is of about the same magnitude. Even if all the ethylene in the bananas were lost, however (about 10 per cent. of the dose), the analyses would still show complete inhibition of ethylene emission during active ripening. Only disappearance of ethylene within the fruit could account for this, since the ethylene emitted by untreated fruits is far more than sufficient to raise the internal concentration to a level equal to that of the external treatment.

It must therefore follow that, since the ethylene does not escape from the bell-jar, it must either be consumed by the fruit or bound by it in some manner. There are strong objections to the latter hypothesis. During the



first day, much ethylene diffused out into the air space; therefore, any adsorbent for ethylene present in unripe fruit must have become saturated during the first day. Following the same line of reasoning, one is eventually driven to the conclusion that only some transient product of the ripening process could be involved, and the rise in ethylene emission when the ripening process becomes complete is hardly sufficient to account for all of the ethylene which must have been bound during ripening. Ethylene therefore must disappear by chemical change during ripening.

Although both respiratory and hydrolytic processes go on at a high rate during ripening, there is reason to believe that ethylene is consumed in the latter processes rather than in the former. In neither graph (fig. 2) is there any evidence of a direct relationship between ethylene consumption and respiratory activity. It is true in a general way that ethylene consumption is great when respiration is high, but widely different respiratory rates may correspond to the same degree of ethylene consumption.

WOLFE (5) has investigated the rate of hydrolytic processes during banana ripening. He finds that the rate of hydrolysis of starch plotted against time gives a somewhat symmetrical curve with a maximum at about the same time as the respiratory maximum. Evidently such a curve resembles much more closely the ethylene consumption curve.

Even more significant is the observation of WOLFE that while ethylene treatment caused little change in the total respiratory activity over the ripening period, it did produce consistent increases in the rate of starch hydrolysis.

All the evidence at hand points, therefore, to a connection of ethylene with the hydrolytic rather than the respiratory processes in ripening bananas, although it may act also as a stimulator of the latter.

Nothing is known at present which suggests a mechanism for the stimulation of starch hydrolysis by ethylene, or its consumption therein. REA and MULLINIX (3) state that ethylene is able of itself to act as a catalyst for the hydrolysis of starch. The author has repeated this work, with a modification. REA and MULLINIX measured the extent of hydrolysis of a starch suspension which had been saturated with ethylene, by a method involving the reduction of Fehling's solution. It was considered that hot alkali was a rather drastic treatment to which to subject starch, and the hypoiodite method of WILLSTÄTTER and SCHUDEL (4) was selected to determine the amount of hydrolysis, on the basis that the possibility of side reactions was less. Under these conditions no hydrolysis of corn starch with or without ethylene was detectable at the end of a week. If a comparison of the two experiments is justifiable, it might be concluded that in the work of REA and MULLINIX ethylene had rendered the starch more susceptible to alkaline hydrolysis. In any case it may be said that ethylene alone cannot cause

the hydrolysis of starch. Nor does ethylene have any influence on the saccharifying activity of taka-diasase according to ENGLIS and ZANNIS (1).

The results of the present experiment explain the acceleration by ethylene of the ripening of fruits which are already producing considerable amounts of ethylene, and confirm the previously offered explanation for the shape of the ethylene emission curve for ripening bananas.

### Conclusions

Ethylene is consumed by the bananas in the course of the ripening process, probably in connection with the hydrolysis of starch. Ethylene treatment of fruits already producing ethylene is believed to accelerate ripening because of this fact.

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# EVIDENCE FOR THE ESSENTIALITY OF SILICON FOR GROWTH OF THE BEET PLANT<sup>1</sup>

G. J. RALEIGH

(WITH ONE FIGURE)

## Introduction

In experiments as yet unpublished, difficulty was encountered in growing plants of the Detroit Dark Red variety of the table beet (*Beta vulgaris* L.) in culture solutions of low concentration in asphalt-painted iron containers. When the plants were transferred from solutions made with a distilled water contaminated with tap water to solutions made with uncontaminated distilled water, the plants in lower concentrations of nutrient salts wilted badly within a few days after being transferred, and the roots became dark in color. Those in higher concentrations made satisfactory growth, which suggested the possibility that some necessary element was being supplied as an impurity in the C.P.-grade chemicals. Preliminary experiments with varying quantities of the four chemicals employed in making the solutions indicated that the impurities might be contained primarily in the ammonium phosphate.

Since A5 (B, Cu, Mn, Zn, Mo) and B6 (Ti, V, Cr, W, Co, Ni) chemicals<sup>2</sup> had been supplied, it was thought possible that the missing nutrient might be one contained in the C13 mixture used by ARNON (1) (Al, As, Cd, Sr, Hg, Pb, Li, Rb, Br, I, F, Se, Be). Single plants from the concentrated solutions were transferred to Mason jars coated with a thin layer of asphalt paint and containing nutrient solutions one-half as concentrated as those used by ARNON. The plants were supplied with various combinations of the 13 micro-elements. Others received small quantities of sodium, chlorine, and increased quantities of B, Cu, Mn, Zn, V, Cr, W, Co, and Ni.

The solutions were not aerated. After a few days the plants wilted irrespective of treatment. The possibility existed that beet plants might have a high requirement for oxygen in the solution and for that reason these plants were kept for comparison with those grown in metal containers in aerated solutions. After 3 weeks the roots of the plants in the Mason jars were making good growth and were lighter in color than any of those in the metal containers. This observation suggested that some nutrient was dif-

<sup>1</sup> The writer is especially grateful to Professor D. R. HOAGLAND for making the facilities of the Division of Plant Nutrition available for the work and for his interest and suggestions, to Mr. P. R. STOUT for suggesting the method and preparing the  $K_2SiO_3$  used in experiment 4, and to Drs. MAX GARDNER and C. M. TOMPKINS for suggestions concerning the control of damping-off of the seedlings in sand. This work was done while on sabbatic leave from Cornell University.

<sup>2</sup> For the composition of similar A and B supplementary solutions see citation (1).

fusing from the soft glass through the asphalt coating. Trial experiments were set up using 10 p.p.m. of Si as  $K_2SiO_3$  and aluminum as aluminum sulphate at the rate of 1 p.p.m. and of 0.5 p.p.m.—much in excess of the amount of that element supplied in the C13 chemicals. After several days all of the plants except those supplied with silicon wilted as previously described. Other experiments were conducted in unpainted Pyrex beakers. Plants not supplied with Si were markedly checked in growth during the first 10 days. Later the difference in growth rate between these plants and those receiving Si disappeared, probably owing to Si dissolved from the glass by the solution. These experiments indicated that slow growth without Si was not associated with the asphalt paint used on the iron containers.

On the basis of these suggestions a series of experiments was carried out to study the relation of Si to the growth of the beet plant. In order to avoid the added impurities in more concentrated solutions the plants were grown in dilute solutions of the following composition:  $NH_4H_2PO_4$ , 0.0005 M;  $MgSO_4$ , 0.001 M;  $Ca(NO_3)_2$ , 0.002 M;  $KNO_3$ , 0.003 M. There is a large amount of evidence (2) that such solutions give as good results as more concentrated solutions if the plants are not grown for long periods without changing the solutions.

In all cases the solutions were made from the dry, C.P.-grade salts immediately before use. A and B mixtures of micro-elements were added to supply elements which are known to be required as well as others which may possibly have a function in plant nutrition. When silicon was supplied its source, except in experiment 4, was pure grade  $K_2SiO_3$ . Iron was added three times weekly. In all experiments an attempt was made to maintain the pH of the solutions between 6.1 and 6.5.

Seed was treated with 3 per cent. formalin for 10 minutes and planted in coarse sand. Except where indicated the experiments were carried out in asphalt-painted 15-liter iron containers supplied with pin-punctured rubber tube aerators and asphalt-painted plaster of Paris tops.

### Procedure

#### EXPERIMENTS 1 AND 2

PLANTS GROWN IN SOLUTIONS WITH SILICON BEFORE EXPERIMENTATION.—Seed was sown December 22, and 10 seedlings for each container were transferred to solutions containing 10 p.p.m. Si on January 9. On January 17, when the plants averaged 3.6 cm. in height, they were changed to solutions without silicon and solutions which had a concentration of 17 p.p.m. Si. Data given in table I were taken February 13.

Other plants of the same lot were grown in solutions containing 10 p.p.m. Si until February 17 when they were 17 cm. in height before being transferred to solutions with 15 p.p.m. Si and to others without Si. Six plants

were set out in each container. They were harvested March 11. Results are given in table I.

TABLE I  
EFFECT OF ADDING SILICON ON THE GROWTH OF BEETS IN CULTURE SOLUTIONS.  
AVERAGE FRESH WEIGHT IN GRAMS

TREATMENT	TOPS	BEETS	ROOTS CENTRIFUGED
	gm.	gm.	gm.
Experiment 1			
with Si 1 .....	3.15	.....	0.85
with Si 2 .....	3.24	.....	0.80
without Si 1 .....	0.99	.....	0.46
without Si 2 .....	0.63	.....	0.34
Experiment 2			
with Si 1 .....	32.78	9.05	5.92
with Si 2 .....	35.02	12.87	6.36
without Si 1 .....	11.22	3.68	3.31
without Si 2 .....	7.70	3.12	2.90
Experiment 3			
with Si 1 .....	2.51	.....	0.51
with Si 2 .....	2.20	.....	0.48
without Si 1 .....	0.26	.....	0.11
without Si 2 .....	0.28	.....	0.10
Experiment 4			
with Si 1 .....	1.55	.....	0.28
with Si 2 .....	1.15	.....	0.21
without Si 1 .....	0.36	.....	0.10
without Si 2 .....	0.25	.....	0.06

#### EXPERIMENT 3

YOUNG PLANTS.—Seed was sown January 7, and 22 plants to the container were transferred to experimental solutions on January 20, as soon as they had reached sufficient size to make the transfer practicable. At that time the cotyledons had a spread of 2 cm. A total of 19 p.p.m. Si in the nutrient solution was supplied to those plants which received silicon. The plants were harvested February 20 with results as shown in table I (see also fig. 1).

#### EXPERIMENT 4

PURIFIED SILICON.—Since the  $K_2SiO_3$  used in experiments 1, 2, and 3 might have contained impurities, the work was repeated using  $K_2SiO_3$  prepared by distilling off  $SiF_4$  produced by the reaction of  $HF$  on amorphous  $SiO_2$ , hydrolyzing the  $SiF_4$  in water, neutralizing with  $NH_4OH$ , and filtering off a large part of the  $F$  as  $NH_4F$ . The remaining  $F$  was removed by heating to  $850^\circ C$ . The residual  $SiO_2$  was weighed and converted to  $K_2SiO_3$  by heating with the equivalent amount of  $K_2CO_3$ . The  $SiF_4$  was collected



in platinum ware. All parts of the procedure beyond that point were carried on in platinum.

Plants of the same lot as those used in experiment 3 were transferred on January 24 to solutions containing 10 p.p.m. Si and on February 4 when they averaged 4 cm. in height were again shifted, some to solutions without Si and others to solutions with 8.3 p.p.m. Si furnished by the purified  $K_2SiO_3$ . The plants were harvested February 18. Although the period of the experiment was short, the differences in the weights of plants grown with silicon and those without were marked as shown by the data in table I.

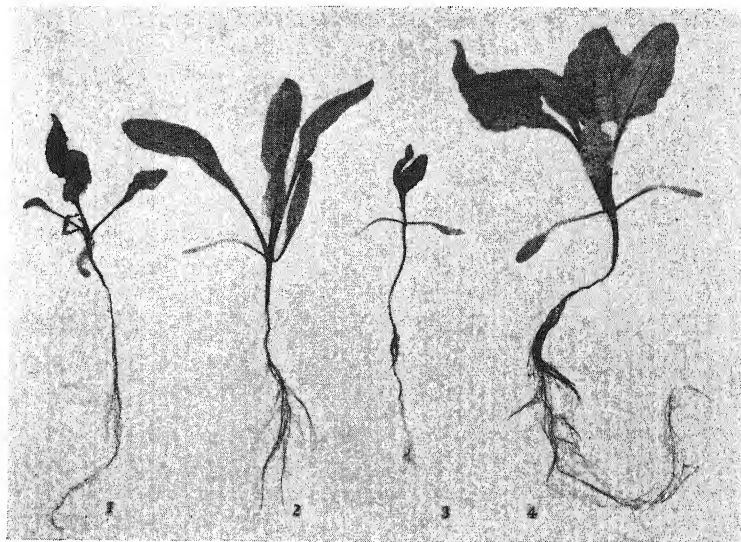


FIG. 1. Plants grown with and without Si in culture solutions. One and 3 without Si, 2 and 4 with Si; 1 and 2 from experiment 4, 3 and 4 from experiment 3.

#### SYMPTOMS OF SILICON DEFICIENCY IN THE BEET

The response to the silicon used in experiments 1, 2, and 3 and to that supplied in experiment 4 was apparently the same. Plants made rapid growth with green foliage (including the cotyledons), and straw colored roots. Plants in solutions deficient in silicon made very slow growth. After about 3 days in such solutions the roots became slightly darker in color and after a considerable time very dark. At this stage the roots were often covered with a growth of organisms, probably owing to the food supplied by the dying roots. On older beets secondary growth of roots was common. When plants were transferred to silicon-deficient solutions at an early stage, damping-off was common after a considerable time in the solutions. When silicon was added, practically no damping-off occurred.

As indicated previously, wilting of the outer leaves during periods of high transpiration was one of the early symptoms of silicon deficiency. These leaves developed anthocyanin along the veins including the secondary veins. In younger plants the cotyledons turned yellow and in most cases soon died.

### Discussion

A large amount of work has been done regarding the essential nature of silicon and its possible relation to phosphorus in the nutrition of plants. SOMMER (5) improved the growth of rice and of millet by the addition of silicon to the culture solution. LIPMAN (4) found that sunflower and barley were definitely benefited, especially as regards seed production by the presence of silicon in the culture medium. ISHIBASHI (3) reported increased growth of rice grown in pots when air-dried silicic acid was added to the soil. In a previous experiment he obtained increased yield of rice in water culture when Si was added to the nutrient solution.

The present investigation indicates that silicon plays an important rôle in the growth of the table beet in culture solutions, and in fact it seems to be indispensable. That the response to silicon in these experiments was more marked than that reported by earlier investigators may be explained on the assumption that the beet has a higher requirement for Si than plants previously studied. Possibly also the chemicals used in some of the earlier experiments may have contained considerable silicon as an impurity. In other cases the coatings of the glass containers may have allowed silicon to diffuse from the containers into the solutions.

Under some conditions phosphoric acid reacts with silicon compounds. In this work the ammonium phosphate carried a major part of the silicon impurities. Conceivably in some of the experiments in which silicon was beneficial when used with very low quantities of phosphorus in the nutrient solution and the benefit attributed to the influence of Si on the utilization of phosphate by the plant, the response was in reality a direct response to silicon.

### Summary

Experiments are reported which indicate that silicon is an indispensable chemical element for growth of the beet plant.

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## BRIEF PAPERS

### A METERING PUMP FOR SMALL VOLUMES OF SOLUTION

M. A. RAINES

(WITH ONE FIGURE)

The method usually employed in botanical laboratories for supplying a regulated flow of solution to a culture vessel is by the use of a capillary resistance device, as summarized by ZINZADZÉ (3). Another method, recently employed by the writer (1), is by the use of wicks disposed over the edge of a constant level tank. Both methods are extremely simple. Many workers, however, find them quite onerous on account of the developed skill and dexterity required for adjusting the rate of the solution flow, and the liability to decrease or stoppage of flow owing to the clogging of the small orifices or fine capillary channels employed. For such workers the use of a metering pump is indicated.

The metering pump here described was devised for use in connection with the modified set-up for wick culture (2). It is believed that as described, or in modified form, it will probably be found advantageous also in other situations in the laboratory. It will be noted that the pump has only one moving part. There are no valves nor tripping mechanism which may get stuck, and no small orifices which may become clogged. The solution, in its path through the apparatus, need not come into contact with any material other than glass.

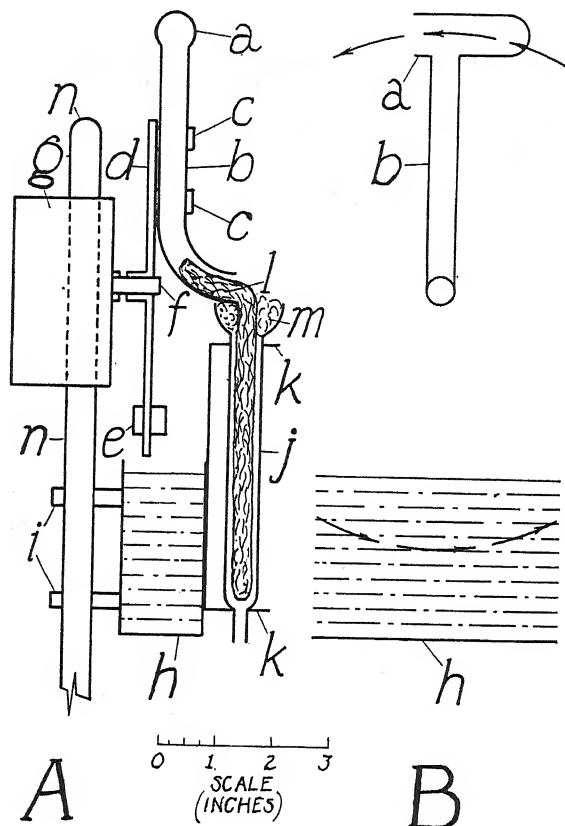
The essential features of construction of the apparatus are illustrated diagrammatically in the accompanying figure, in which A is a view from the side, showing details of the entire assembly, and B a representation as seen from the front of only the measuring cup (a) with its outflow tube (b) and the constant level tank (h). The arrows in B indicate the path of travel of the measuring cup—through a circular path in a vertical plane. At the bottom of this path the cup is dipped into the solution contained in the constant level tank (h), and towards the top of the path the liquid contained in the cup drains down the outflow tube (b) and out its open end into the funnel tube (j), from which it flows into the culture vessel below (not shown).

The pump is driven by a small synchronous electric motor (g)<sup>1</sup> mounted by means of clamps (not shown) on the rod (n) of a laboratory stand. This motor is sufficiently powerful to handle measuring cups of a capacity up to 10 ml. The capacity of the cup may be reduced any desired amount by the simple expedient of inserting wads of glass wool into it. Regulation of rate

<sup>1</sup> A "time machine" obtained from the Hansen Manufacturing Co., of Princeton, Indiana. These small motors cost less than \$2.00 each, and are obtainable from stock in a variety of set speeds from  $4\frac{1}{2}$  revolutions per minute to 1 revolution per day.

of flow of solution is also accomplished by substituting motors of different speed.

As the open end of the outflow tube is directed horizontally, the solution often does not drain out of it completely. This difficulty may be obviated by introducing a wick (1)<sup>2</sup> of spun glass into the open end of the outflow tube,



the lower end of the wick being suspended in the funnel tube (j). Wads of glass wool (m) at the upper end of the funnel tube are used to hold the wick in place. Such a glass wick operates excellently to effect complete drainage of solution from the outflow tube. Its use facilitates compactness of construction of the apparatus, and precision of delivery of the measured quantity of solution (especially important when a measuring cup of small capacity, as 0.5 or 1 ml., is being used).

The measuring cup (a) with its outflow tube (b) is attached by means of

<sup>2</sup> Made from strands of glass rope obtained from the Plymouth Cordage Co., North Plymouth, Mass.

spring clips (fuse clips) to the carrier arm (d) which is mounted on the motor shaft (f). In mounting the measuring cup on the carrier arm, it is desirable that the open end of the outflow tube be well centered, in line with the motor shaft. At the other end of the carrier arm is an adjustable counterweight (e). The funnel tube (j) rests on brackets (k) attached to the wall of the constant level tank (h). This in turn is mounted on the rod (n) of the laboratory stand by means of the clamps (i).

### Summary

A bucket type pump is described for delivering small measured volumes of solution. It has only one moving part, and no small orifices. A glass wick may be used for draining the solution from the open end of the outflow tube. The solution, in its path through the pump, need not come into contact with any material other than glass.

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METERING PUMP AND TURNTABLE ARRANGEMENT FOR  
SUPPLYING MEASURED QUANTITIES OF NUTRIENT  
SOLUTION SUCCESSIVELY TO ANY NUMBER OF  
CULTURE VESSELS<sup>1</sup>

H. G. DU BUY

(WITH FIVE FIGURES)

In connection with experiments in plant nutrition, especially the plant physiological worker frequently has the task of supplying definite quantities of solution, in a given time, to each of a number of culture vessels. The apparatus here described was constructed to effect this in the case of an experiment in which the plants are being grown in sand in stoneware jars. Each experimental set consists of 12 jars, intended to receive equal quantities of the same nutrient solution. The 12 jars of a set are placed on a wooden bench.

The new arrangement replaces a capillary drip method previously employed. The change in method has effected a considerable saving in time required for adjusting and servicing the supplying of nutrient solution to the culture jars. It is recognized that there are individual differences of facility and dexterity in laboratory techniques, and also that preferences in, and successes with, laboratory procedures are not without a subjective element.

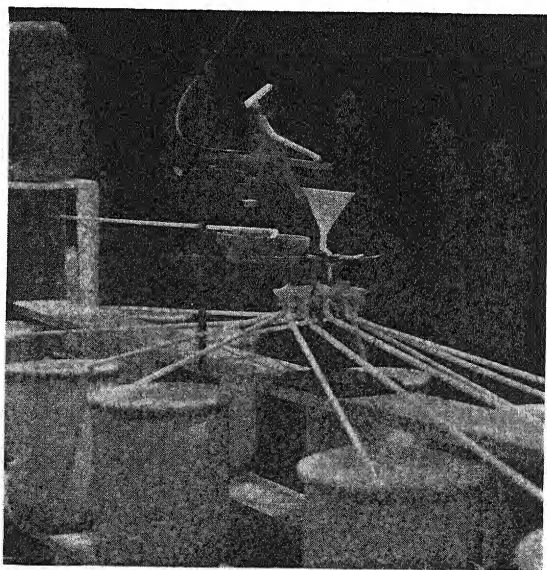


FIG. 1. Photograph of the apparatus in use.

<sup>1</sup> Contribution no. 496, Department of Botany, the University of Maryland.

## Solution measuring mechanism

This is an adaptation to the present situation of the metering pump for small volumes of solution devised by RAINES<sup>2</sup> of Howard University in connection with the modified set-up for wick culture developed by him and described in the preceding paper. Figure 1 is a photograph of the entire mechanism, and figure 2 a front view. The motor (m, fig. 3) turns a cup (a),

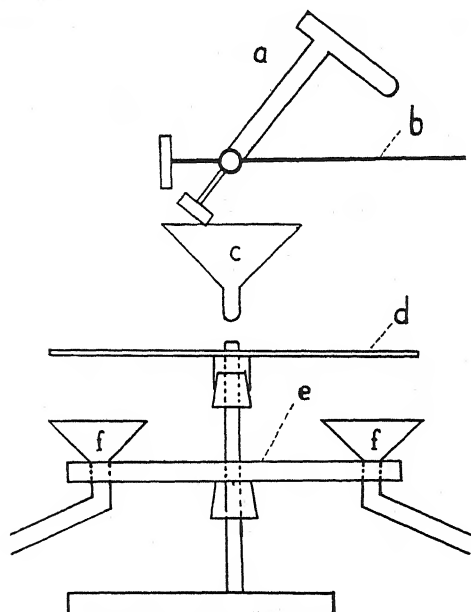


FIG. 2. Front view of the whole mechanism.

which, due to the gradual slope of the outlet tube, deposits the water in a receiver funnel (e). The gradual slope of the cup tube foregoes the use of a wick in order to drain the tube, and also the precise centering of the outlet.

Figure 3 shows a side view. Container (v) is connected by glass tubing with a reservoir (not shown in fig. 3) which serves as a constant level device. For instance, a turned-over 5-gallon carboy with trough can be placed at any suitable point and the liquid from there administered to the container. The water of the cup drops into a receiver.

This modified metering pump has been used in connection with a turntable and a funnel system instead of a receiver with a wick system which was unsatisfactory in the use of nutrient solutions.

<sup>2</sup> Raines, M. A. *Plant Physiol.* 14: 829-831. 1939.

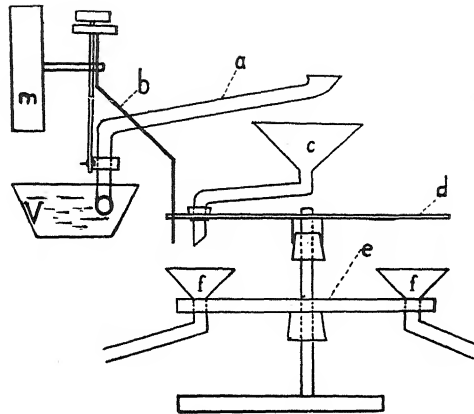


FIG. 3. Side view of the mechanism.

#### The distributor mechanism

The receiver funnel (c) (fig. 4) is attached to a turntable (d). This turntable, which turns on a metal bearing, has as many dents as there are

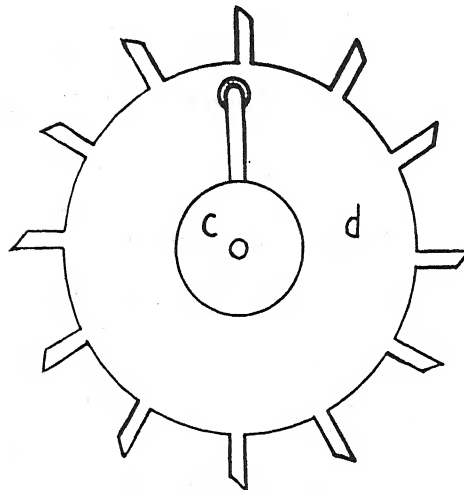


FIG. 4. Turntable with distributing funnel.

cultures to supply. Each dent catches the turn axis (b) which is turned by the same motor that turns cup (a). By this means the outlet of receiver funnel (c) is moved serially on top of each small funnel ( $f^1$ ,  $f^2$ ,  $f^3$ , etc.) of funnel table (e) (fig. 5) which is stationary. The funnels supply the liquid to the cultures. They can be easily removed from the slits ( $g^1$ ,  $g^2$ ,  $g^3$ , etc.) of funnel table (e).



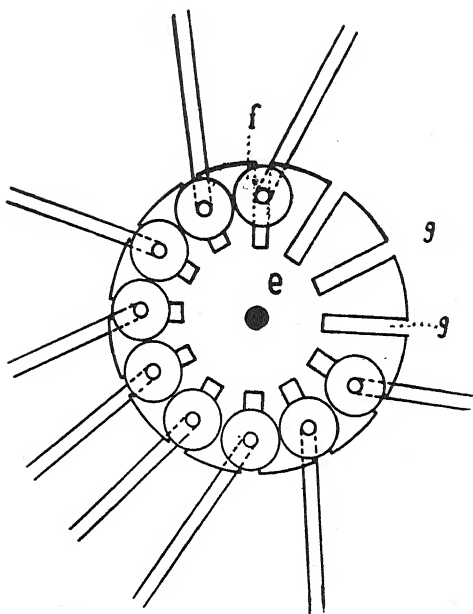


FIG. 5. Funnel table with notches and some secondary funnels going to the jars.

The operation of the apparatus is made more clear in the figures. Except for the motor all parts are made of easily obtainable material: the turntable (fig. 4), as well as the turning axis, is made of galvanized iron. Standard ring stands are used. In order to vary the height of the distributor, a secondary ring-stand rod may be used for a carrier and may be made adjustable by attaching this rod to the ring stand with a clamp. The metal bearing may be made of the brass part of a fuse and attached to a cork. The funnel table may be made of wood or Celotex. The glass parts can be painted in order to prevent development of algae.

The advantages of the apparatus are:

1. The water or culture solution comes into contact only with glass.
2. The amount of liquid to each jar is well defined and can be varied at will.
3. The wide tubing used in the whole system prevents all clogging even after long periods.
4. All glass parts may be easily removed for cleaning and are easily adjustable.
5. Since only the constant level reservoir has to be filled after long intervals, the method is not time consuming.

UNIVERSITY OF MARYLAND  
COLLEGE PARK, MARYLAND

CONVENIENT SEEDLING SUPPORT FOR GROWING PLANTS  
IN WATER CULTURE<sup>1</sup>

STUART DUNN

(WITH ONE FIGURE)

Difficulties are frequently encountered with the usual method of starting seedlings in water culture for nutrient solution studies. This is conventionally done by placing the sterilized and soaked seeds on a paraffin-impregnated mosquito netting stretched tightly over the top of a jar filled with either water or dilute nutrient solution. After the seedlings have reached a convenient size (about 2 or 3 inches high, if wheat is being used), they are transferred to a support. This support may be of stiff paraffined paper with holes punched in it, or similarly treated thin cork stoppers provided with holes. The seedlings are placed in the holes, supported by a little cotton.

The procedure of transplanting seedlings to these supports has often seemed to hinder the growth of the plants especially when the operations are performed by elementary students in the laboratory, or even by those with more experience. The delicate seedlings are apparently very easily injured at this stage of growth.

A method which avoids this difficulty has proved very successful in class work at the University of New Hampshire. The main features of the method are shown in figure 1. The container may be an ordinary quart fruit

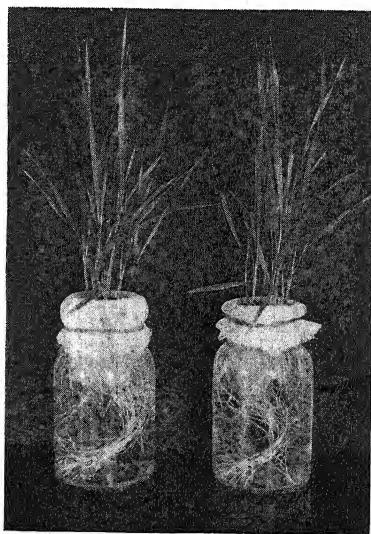


FIG. 1. Seedling support for nutrient culture work.

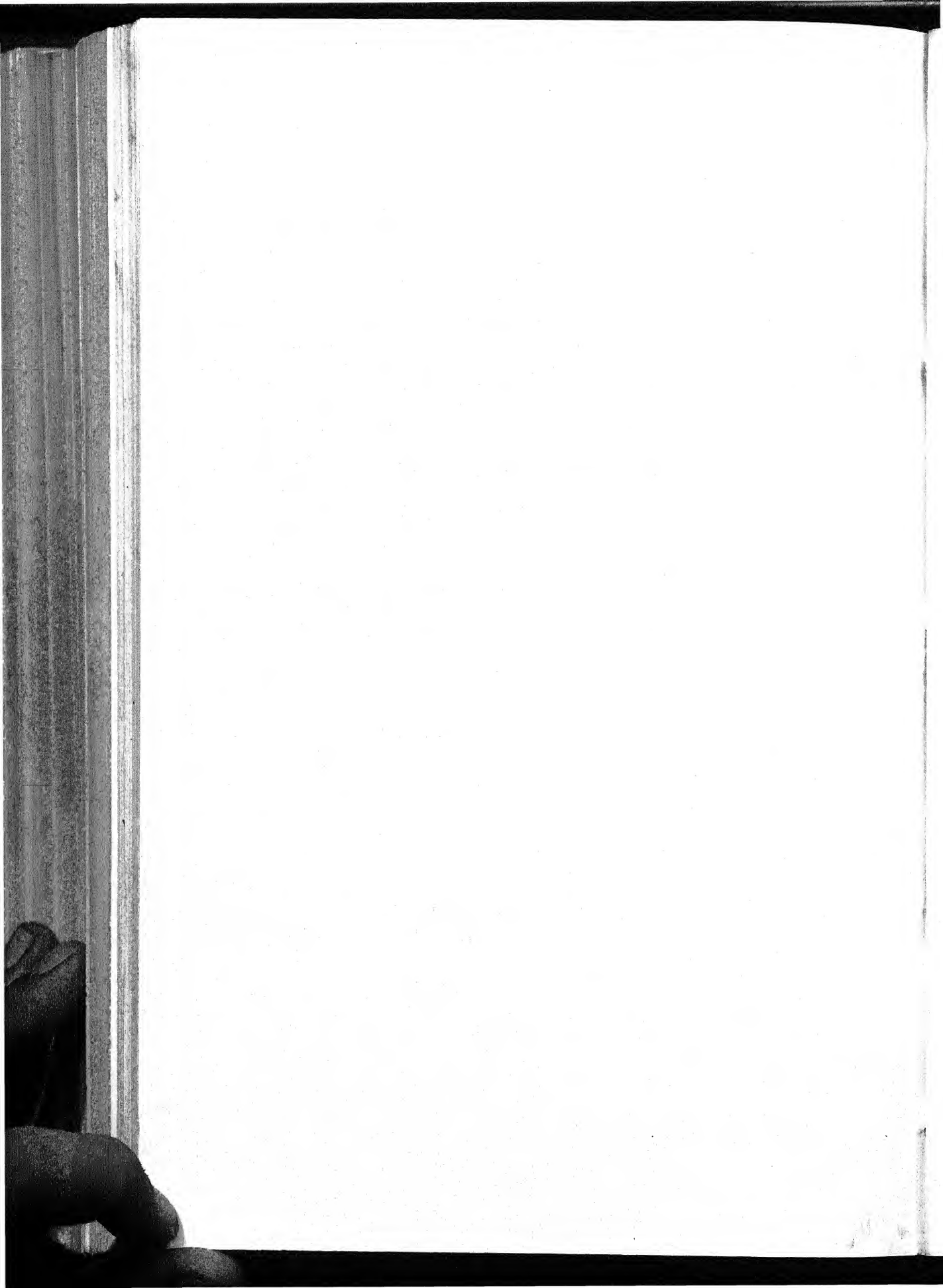
<sup>1</sup> New Hampshire Agricultural Experiment Station scientific contribution no. 73.



jar. The support is made from a circular piece of mosquito netting with a diameter about 6 inches greater than the mouth of the jar. This mosquito netting is impregnated with hot paraffin to prevent molding, and is then fastened over the mouth of the jar by means of a rubber band, with the support adjusted to form a pocket about 1.5 inches in diameter, which dips into the jar. The pocket is filled with washed sand of particles large enough not to pass through the netting. The seeds, previously selected for plumpness, are sterilized by soaking in formaldehyde solution, and allowed to remain in running water overnight. They are then planted directly in the sand, near the surface. The base of the pocket is kept immersed in the nutrient solution at least until the seedlings have grown roots well through the sand into the solution. Changing of the solution is easily accomplished by first drawing off the solution in the jar by suction through a narrow-bore glass tube. This may be thrust between the meshes of the netting at one side, first making a place for it by a pencil point. The fresh solution may then be poured directly through the sand. The method might be adapted for very exact studies by using even more inert material than sand particles such as glass beads or quartz.

The plants shown in figure 1 subsequently grew to the heading-out size with very little attention.

UNIVERSITY OF NEW HAMPSHIRE  
DURHAM, NEW HAMPSHIRE



## NOTES

**Columbus Meeting.**—The sixteenth annual meeting of the American Society of Plant Physiologists will be held in Columbus, Ohio, during the week of December 25 to 30, 1939. The sessions will be held beginning on Wednesday, December 27, and extending through December 30. This change from the usual Tuesday opening is necessitated by the occurrence of Christmas day on Monday.

The headquarters of the Society will be the Neil House, Columbus, and the annual dinner, scheduled for Thursday evening, December 28, will be held in the Junior Ballroom of the Neil House.

The various sessions and symposia will be held in buildings of the Ohio State University, with possible exception of the joint session with Section G, A. A. A. S. The various meetings of physiologists will be arranged in adjacent buildings, so that transfer from one program to another will be expedited. It is hoped, of course, that the grouping of papers, as at Richmond, may be continued. The wisdom of parallel programs of selected non-competing papers was fully demonstrated last year.

The central location of the meeting should make possible a very large attendance. Reservations should be made early, for this meeting is likely to set a record in attendance. For hotel and railway rates, consult the notices in *Science*.

**Western Section.**—The annual meeting of the Western Section of the American Society of Plant Physiologists was held at Stanford University, California, from June 28 to July 1, 1939. Three important symposia were the major features of the meeting: (1) Availability of nutrients in soils to plants; (2) translocation of solutes in plants; and (3) growth. All of these symposia were well attended.

In the symposium on translocation, Dr. K. ESAU discussed the anatomy of vascular tissues, using slides of photomicrographs that were exceptionally good. Dr. C. W. BENNETT discussed the translocation of viruses in plants. Viruses in general move with the food. Dr. D. R. HOAGLAND and Dr. J. P. BENNETT reported on experiments in which they used radioactive elements, which, among other things, very clearly showed that the major salt movement is through the wood, and that considerable lateral movement takes place from wood to bark. The various concepts of the mechanism of translocation of organic materials were discussed in papers by Drs. O. F. CURTIS (read by Dr. J. R. FURR), F. W. WENT, and A. S. CRAFTS. In the extensive discussion that followed, the conclusion was reached that one must distinguish between the four following types of translocation: (1) The "upward movement"

which takes place through the wood, and is mainly caused by transpiration; (2) the "downward movement" which takes place through the sieve tubes, and is caused by pressure flow; (3) movement from cell to cell, in which protoplasmic streaming may aid the diffusion through plasmodesmata; and (4) polar movement of auxin, which possibly takes place along interfaces.

The symposium on growth began with a discussion by Dr. A. S. FOSTER of the morphological aspects of meristematic tissues. Dr. A. R. DAVIS reported on the nutritional factors in growth, in which he especially stressed accumulation and the essential elements. Dr. J. BONNER discussed the rôle of vitamin B<sub>1</sub> in plants, which is now considered to be an important phytohormone. Dr. J. VAN OVERBEEK discussed some of the many aspects of the rôle of auxin in plant growth.

Of the submitted papers, those of Drs. D. R. HOAGLAND, D. I. ARNON, P. R. STOUT, T. C. BROYER, O. BIDDULPH and others on radioactive elements as indicators of accumulation, movement, distribution, etc., were of outstanding interest.

The officers elected for the coming year are: Chairman, Dr. W. M. ATWOOD, Oregon State College; vice-chairman, Dr. A. S. CRAFTS, University of California, Davis; and secretary, Dr. J. VAN OVERBEEK, the California Institute of Technology.

**Life Membership Committee.**—Following the constitutional regulations governing the CHARLES REID BARNES life membership awards, President J. W. SHIVE has appointed a committee whose duty and privilege it will be to select the sixteenth recipient of this award. The committee is composed of the following members: Dr. R. P. HIBBARD, Michigan State College; Dr. PHILIP R. WHITE, The Rockefeller Institute for Medical Research, Princeton; Dr. GEORGE W. PUCHER, Connecticut Agricultural Experiment Station; Dr. GEORGE P. BURNS, the University of Vermont; and Dr. EARL S. JOHNSTON, the Smithsonian Institution, chairman of the committee. It has been customary for the president to announce the decision of the committee and to make the award at the time of the annual dinner. No one will want to miss being present when the life membership is awarded, for these occasions have become notable events in our Society history. Annual dinner tickets will be available at the registration room of the A. A. S.

**Chemical Methods Report.**—Owing to unforeseen difficulties, it has been necessary to postpone for one quarter, the publication of the report of the chemical methods committee which was planned for October, 1939. The report will be placed in the January, 1940, number of PLANT PHYSIOLOGY.

**Errata.**—At the close of the table of contents of volume 14, a small

number of errors, reported by authors, have been corrected. Readers are again urged to record these marginally at the places where the errors occur. In this way the errors become harmless. We wish to express our sincere appreciation to the too few authors who have been thoughtful enough to assist us in detecting some of the mistakes. We wish that all authors would proof-read the finished paper, and report, even when they find no errors. The constant press of future requirements prevents the editors from performing this function as they should.

**Seventh International Botanical Congress.**—The Seventh International Botanical Congress is to be held in Stockholm, Sweden, during the summer of 1940. The dates selected are from about the 17th to the 25th of July. The Secretary of the Congress is Dr. HUGO OSTVALD, Lantbrukshögskolan, Uppsala. A preliminary program of the Congress may be obtained by addressing the Secretary. The permanent program, it is hoped, may be ready for distribution by the first of the year, 1940. Dr. H. LUNDEGÅRDH is Recorder for the Section on Plant Physiology. He may be addressed also at the Lantbrukshögskolan, Uppsala. It is hoped that conditions may remain favorable for a large attendance at the Congress by American delegates.

**Back Number.**—The editor has received a rare number of *PLANT PHYSIOLOGY* from a member of the Society, who offers it for sale at \$2.50, the usual price for current single numbers. The number is volume 1, no. 3, July, 1926. This volume has been out of print for many years. Here is an opportunity to complete a broken file, if you lack this number.

**Lost Numbers.**—It has been customary to supply free of charge a copy of any number of *PLANT PHYSIOLOGY* to members and subscribers in case of nondelivery for any cause. In view of present conditions throughout the world, it is not possible to guarantee delivery. The edition of the journal is limited, of course, by our funds for publication; in case of serious losses of mail addressed to foreign countries, as, for instance, on ships at sea, it would be impossible to replace the lost copies. We will make every effort to deliver the issues to all members and subscribers, but cannot assume responsibility to replace lost copies under present world conditions. This does not apply at present to domestic subscribers and members of the American Society of Plant Physiologists.

**Harold Mestre.**—In the death of Dr. HAROLD MESTRE on September 9, 1939, at the age of 55 years, the American Society of Plant Physiologists has lost one of its devoted members. Dr. MESTRE was usually present at the



annual meetings, and often took part in animated and thoughtful discussion of the problems that fell in his own field of interest. He will be greatly missed by his colleagues and friends.

Born at Mamaroneck, New York, on August 16, 1884, MESTRE entered collegiate training with much more than the usual maturity. He sought his higher training in California, where, at the age of 39, he received from the University of California, his degree of Bachelor of Arts. He became a teaching fellow and assistant in zoology at the University of California after his graduation, and for several years was assistant and associate professor of biology at the California State Teachers College at Fresno. Entering Stanford University as a fellow in 1927, he pursued his graduate work intensively, and received his Ph.D. degree in 1929. After receiving his degree he was acting assistant professor and assistant professor of biophysics at Stanford for four years. In 1934 he entered Yale University as an honorary fellow in the Medical School. During the last two years he had been at Bard College, Columbia University, at Annandale-on-Hudson. Here he was professor of biophysics, director of studies, and in 1938 became dean of the college.

His main contributions were in the field of radiation, with special reference to spectrophotometry of leaves and algal cultures as related to photosynthesis, photometrical studies of bacterial suspensions, etc. His death is a distinct loss to American botany, particularly to plant physiology, because there are too few accomplished biophysicists in the field of the plant sciences. The memory of his accomplishments will remain as an inspiration to others who may be struggling to advance in the same field.

**Advances in the Chemistry of Natural Organic Substances.**—The second volume of the *Fortschritte der Chemie organischer Naturstoffe* has been published by Julius Springer, Wien. The general sponsorship of this series of reviews was given in the January, 1939, number of PLANT PHYSIOLOGY. The second volume contains nine reviews of great interest to all biochemical investigators. The first review concerns lignin, which is discussed by K. FREUDENBERG, whose investigations in this field have been numerous and valuable. His short summary is especially interesting to the general student.

Lichen substances are reviewed by Y. ASAHINA. Five groups of compounds are included; the fatty materials, aromatic derivatives of the benzol series, depsides, depsidones, and anthraquinone derivatives.

The flavins are monographed by H. RUDY, most of the attention being given to lactoflavin. A general discussion is followed by synthetic procedures, and a discussion of the co-enzyme function of flavins.

Other reviews include the chemistry of the iodine compounds of the thyroid, by C. R. HARRINGTON; the structure and synthesis of vitamin C

(ascorbic acid) and its analogues, by E. L. HIRST; recent methods of oligosaccharide syntheses, by G. ZEMPLÉN; chitin and its decomposition products, by G. TÓTH; tobacco alkaloids, by E. SPÄTH and F. KUFFNER; and spectrochemistry of fluorescence in the study of biological products, by CH. DHÉREÉ. Six of the papers are in German, two in English, and one in French.

These reviews are well prepared, and thoroughly up to date. They are most valuable in giving the reader a general, yet specific, survey of the recent progress in biochemistry of the substances reviewed. The progress of recent years has been so rapid, and so far reaching, that without such integrating discussions one loses the general significance of research in a maze of details. The price of volume 2 of the *Fortschritte* in cardboard binding is RM. 28. Orders should be sent to the publishers, in Vienna.

**Growth and Movement.**—The second volume of a *Lehrbuch der Pflanzenphysiologie* by E. BÜNNING, K. MOTHES, and F. VON WETTSTEIN, bears the title *Die Physiologie des Wachstums und der Bewegungen*. Dr. E. BÜNNING is the author of this volume. The text falls into three parts, the first of which is a general discussion of rest and activity, the energetics and course of growth, and the mechanisms and catalyzers of extension and plasma building. The second part deals with the physiology of the mechanisms of movement, such as turgor, flagellae, surface energy, plasma streaming, wall tensions, and imbibitional movements. The final section treats of the physiology of stimuli. These include mechanical, radiational, temperature, electrical, gravitational, chemical, and autonomous stimuli and the plant's responses to them. The concluding chapter considers some of the general problems of stimulation and growth response physiology. The discussions are of exceptional interest because of the increasing application of hormone concepts to the explanation of the physiology of growth and movement. The book is profusely illustrated, with 233 text figures. The price of the work bound in paper is RM 18, or RM 19.8 bound in cloth. The publishers, Julius Springer, Linkstrasse 22-24, Berlin W 9, will be pleased to receive orders for this work.

**Earth's Green Mantle.**—A charming volume by SIDNEY MANGHAM, Professor of Botany in University College, Southampton, bears the title *Earth's Green Mantle*. Works suitable to the layman as well as student are relatively few. MANGHAM tells the story of plant life in eleven chapters, describing the origin and development of plants, the tools by which the secrets of life are discovered, such as microscopy and chemical examination, and then the plant is presented as a machine, whose structures and functions are described under such attractive titles as the machine in the making, the machine in action, mass production and new models, and engine troubles.

The final chapter on progress and prospects presents a generous point of view from which the layman may appreciate the spirit of botanical progress. We recommend this work as one which may be used to create enthusiasm for botany among those who have no technical background for it. A reading list offers additional suggestions for the more complete enjoyment of plant life. The Macmillan Co. offer this unusually interesting work at \$3.50 per copy. It is excellent for gift purposes.

**Dictionary.**—A *German-English Science Dictionary* for students of agricultural, biological, and physical sciences has been published by the McGraw-Hill Book Co. The author is LOUIS DE VRIES, Professor of Modern Languages at Iowa State College. This handy volume will be welcomed by many a student who is struggling for mastery of German scientific literature. The only criticism which one can offer to such works is that they are seldom complete enough for all of the requirements; words omitted, or definitions not versatile enough. There are about 48,000 words included, however, which is a very good start. Students who find any inadequacy should accept the author's invitation to submit lists of terms which ought to be included. Just at a chance, the editor looked up Photosynthese as a key word in botany; but although there are a number of Photo-compound words listed, this one was not. Perhaps it was thought to be too easy to need listing. It isn't much easier, however, than Photo-graphie which is listed. A good test will be to use the dictionary on a serious chapter in some recent German text on Pflanzenphysiologie; but keep a pad at hand to list the terms to be added. A later edition can remedy any serious sins of omission. The author has made a fine start at a good, usable dictionary. The quoted price is \$3.00 per copy.



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